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Seasonal variation in mussel byssal thread mechanics

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Summary

The blue mussel, *Mytilus edulis*, attaches itself to the substrate by producing a radially arranged complex of collagenous byssal threads. The strength of byssal attachment, or tenacity, has been shown to vary seasonally on Rhode Island shores, increasing twofold in spring in comparison with fall. It was previously assumed that this seasonality was due to increased thread production following periods of increased wave action; however, recent findings do not support this view. As an alternate hypothesis, this study evaluates the contribution of seasonal changes in the material properties of byssal threads to an annual cycle in mussel attachment strength. Tensile mechanical tests were performed seasonally, on both newly produced threads and on threads outplanted

in the field for up to nine weeks. Threads produced in spring were over 60% stronger and 83% more extensible than threads produced in all other seasons. The mechanical integrity of byssal threads also deteriorated over time in spring and summer. These results suggest that reduced attachment strength in fall reflects the production of inferior quality threads following a period of increased decay. Here, we propose a new scheme where variation in byssal thread material properties, rather than quantity, explains the seasonal pattern in attachment strength observed on Rhode Island shores.

Key words: *Mytilus*, byssus, attachment strength, material properties, tenacity.

Introduction

Mussels are well known for their ability to survive in hydrodynamically stressful environments (Bell and Gosline, 1996; Carrington and Gosline, 2004; Paine, 1974; Suchanek, 1978). In the intertidal zone, waves breaking on the shore create small-scale turbulence superimposed on a directional current, and intense wave action creates extreme hydrodynamic forces, which in turn increase the risk of dislodgment and mortality (Bell and Gosline, 1997; Denny, 1987; Hunt and Scheibling, 2001). The blue mussel, *Mytilus edulis* Linnaeus, withstands these large hydrodynamic forces by tethering itself to the substrate with a byssal complex composed of multiple extracellular collagenous byssal threads that radiate from a central stem. Individual threads are secreted by a gland in the foot and comprise three regions: the corrugated proximal region, the smooth distal region and the terminal adhesive plaque, which attaches each thread to the substrate (Brown, 1952; Waite, 1992). Individual threads are both strong and extensible, and these material properties confer a mechanical toughness that exceeds that of mammalian tendon and other collagenous fibers (Gosline et al., 2002).

The strength of byssal attachment, or tenacity, has been shown to vary spatially and temporally; solitary mussels maintain a stronger attachment in comparison with bed mussels

(Bell and Gosline, 1997), which presumably experience lower wave exposure. Attachment strength also follows an annual cycle in *M. edulis* populations, with twofold variation among seasons (Carrington, 2002; Price, 1980; Price, 1982). In Rhode Island, USA, attachment strength peaks in winter/early spring and is lowest during calm summer months (Carrington, 2002). It has often been suggested that the dynamics of attachment strength reflect the mussels' adaptive response to increased flow; producing a greater number of byssal threads enables mussels to remain attached during wavier conditions (Dolmer and Svane, 1994; Hunt and Scheibling, 2001; Lee et al., 1990; Van Winkle, 1970; Witman and Suchanek, 1984; Young, 1985).

The role of thread production in enhancing mussel attachment, however, has recently been questioned. Moeser et al. (Moeser et al., 2006) examined multiple flow levels and found a curvilinear relationship between flow and thread production, with thread production peaking around 10–14 cm s⁻¹ and decreasing dramatically at higher levels of flow (Moeser et al., 2006). In addition, both laboratory and field experiments indicate that thread production is highest when mussels are most weakly attached (Moeser et al., 2006). These findings indicate that increased thread production due to heightened wave action is not the mechanism controlling the

dynamics of mussel attachment strength, and alternative explanations warrant investigation.

One mechanism that could lead to the observed differences in attachment strength throughout the year is seasonal variation in thread mechanical properties. Byssal threads are composed of collagenous proteins with both silk and elastin domains in the distal and proximal regions, respectively (Qin and Waite, 1995). The presence of both metal chelates and DOPA crosslinks combined with specific oxidizing conditions is necessary to maximize the assembly of the individual protein fibers found in threads (Waite, 2002; Waite et al., 2002). Just as the composition of human hair, a proteinaceous extracellular structure, is altered when humans are malnourished (Rushton, 2002), the structure and adhesion of byssal threads may also vary with fluctuations in food supply and water chemistry (Monahan and Wilker, 2004). Threads are known to reflect the geochemical nature of their environment (Coombs and Keller, 1981). However, these geochemical signatures are metabolically transported to the threads rather than adsorbed onto the surface of the threads (Sun and Waite, 2005).

Several studies have quantified the tensile mechanical properties of individual byssal threads (Bell and Gosline, 1996; Carrington and Gosline, 2004; Lucas et al., 2002; Smeathers and Vincent, 1979). Such tensile testing places an individual byssal thread in tension and extends it until failure, providing both a detailed description of thread behavior and an estimate of breaking force and breaking strain. Breaking force is the maximum force supported by a thread, whereas breaking strain is defined as the total distance a thread extends before failure, divided by the initial thread length. Overall byssus strength increases as the breaking force (strength) and breaking strain (extensibility) of individual threads increase. This latter effect is less intuitive; extensibility allows individual threads to stretch further within the byssal complex to realign and recruit more threads with which to withstand hydrodynamic forces (Bell and Gosline, 1996).

The high extensibility of *M. edulis* threads is largely due to the yield behavior of the distal region before thread failure occurs (Bell and Gosline, 1996; Gosline et al., 2002). As a consequence, mussel byssal threads exhibit a characteristic triphasic force–extension curve: an initial stiff phase, followed by a more extensible yield phase, which is in turn followed by a second stiff phase (Bell and Gosline, 1996; Smeathers and Vincent, 1979). Thus, while the extension provided by the distal region yield does not affect individual thread strength, it greatly enhances overall attachment strength by providing the compliance necessary to distribute load among numerous threads in the byssal complex.

Another mechanism that could potentially affect the seasonality of attachment strength is thread durability or decay (Carrington, 2002). Daily tidal fluctuations can expose intertidal organisms to extreme temperature changes and increased evaporation. Marine bacteria are commonly capable of collagenolytic enzyme production (Merkel et al., 1975). Thus, environmental conditions and/or biofouling activity may degrade the collagenous threads, thereby reducing the

mechanical integrity of the byssal complex as a whole. Indeed, Price suggests that thread strength and stiffness decline significantly after approximately 25 days (Price, 1981). It is not known, however, whether the rate of thread decay varies across seasons.

The present study examines two possible mechanisms that could contribute to the seasonality of attachment strength for mussels in Rhode Island, USA. The tensile mechanical properties of individual byssal threads and the deterioration of field-exposed threads were quantified seasonally. This study demonstrates that thread quality and decay vary seasonally and that these two properties significantly influence attachment strength. It is proposed that the combination of these two properties explains the seasonal cycle of attachment strength in *M. edulis* on Rhode Island shores.

Materials and methods

This study was performed in the laboratory at the University of Rhode Island and in the field on the docks of the Graduate School of Oceanography in Narragansett Bay, RI, USA (41.5°N, 71.4°W). For all experiments, mussels were collected from the rocky intertidal zone at Black Point in Rhode Island Sound (41.4°N, 71.5°W). All statistical analyses were performed using Systat (Richmond, CA, USA).

Thread preparation

For each seasonal experiment, 50 mussels (*Mytilus edulis* L.), approximately 4 cm in length, were collected and all byssal material was removed. The right valve of each mussel was attached to a nylon rod using cyanoacrylate glue and elevated 6.5 mm above a granite slab in a laboratory tank held at seasonally appropriate temperatures (8–20°C) (Moeser, 2004). Mussels were held in the aerated tank under low flow conditions (8 cm s⁻¹) for approximately seven days, until a new byssus was produced. Each mussel was then detached from the nylon rod, and the body of the mussel, including the shell, was removed from the byssal complex (byssal threads and stem), which remained attached to the granite slab.

Because mussel attachment correlates with reproductive condition in *M. edulis* (Carrington, 2002), gonad index (GI) and condition index (CI) were measured for each subject as follows. Shell length was measured with vernier calipers to the nearest mm, and the mantle, including the gonads, was separated from the remaining body tissue. The mantle and the remaining body tissue were then dried to a constant mass at 60°C (1–2 days). GI was calculated as the dry mantle mass divided by the total dry tissue mass (Carrington, 2002). CI was calculated as the total dry tissue mass divided by the shell length cubed, where shell length cubed was used as a proxy for volume (Moeser et al., 2006).

Mechanical testing

A subset of granite slabs (7–9 slabs) was haphazardly chosen for immediate mechanical testing to provide an estimate of initial thread quality (threads 1–7 days old; *N*=15–18 threads).

These 'initial' threads were tested on the following dates: 28 April 2003, 15 July 2003, 24 September 2003 and 26 January 2004, corresponding to spring, summer, fall and winter, respectively. The remaining granite slabs were outplanted to the field and attached to a piling at the Graduate School of Oceanography at a tidal height naturally inhabited by mussels (~0.75 m above MLLW), exposing the remaining byssal complexes to seasonal environmental stresses. Six to eight haphazardly chosen slabs were collected approximately every two weeks to quantify the durability of byssal threads as described below. The spring and fall experiments lasted nine weeks, whereas the other experiments were terminated at six weeks due either to a lack of testable threads (summer) or loss of granite slabs (winter). It should be noted that since the animals were removed from the byssal complexes, the byssal threads may experience reduced mechanical degradation, which could accelerate environmentally induced deterioration in the field.

An Instron 5565 tensometer (Instron, Canton, MA, USA) was used to measure individual thread strength and extensibility following Bell and Gosline (Bell and Gosline, 1996). The plaque of each thread remained attached to the granite slab, which was anchored within a small acrylic tank filled with 15°C seawater. The proximal end of the thread was detached from the stem and mounted within a pair of grips using cardstock and cyanoacrylate glue, which were attached to a mobile crosshead and submerged in seawater. Threads were then extended until they were held taut without accruing any force; thread length (l_0 ; ± 0.02 mm) was then quantified. The crosshead was raised at the standard extension rate of 10 mm min⁻¹, placing an individual thread in tension, until failure occurred; force (± 0.002 N) and extension (± 0.02 mm) were recorded every second.

Thread strength, or breaking force (F_b), was defined as the force needed to induce thread failure. Extensibility, or breaking strain (ϵ_b), was calculated as the extension at failure, divided by the initial thread length (l_0). The yield force (F_y) and yield strain (ϵ_y) were defined as the force and strain values at the intersection of the tangent to the initial portion of the force-length curve and the tangent to the plateau region of the curve (Bell and Gosline, 1996). This point marks the thread transition from high to low stiffness (Smeathers and Vincent, 1979). Both the yield force and strain were determined manually for each thread that failed beyond the plateau phase of the force-extension curve.

Thread breaks that occurred at the grips were assumed to underestimate the actual mechanical properties of an individual thread and were, therefore, discarded from the analysis. Threads that failed at the plaque/granite boundary were retested using the method described above; in retests, the byssal plaque was mounted within a pair of stationary grips using cardstock and cyanoacrylate glue. Each byssal thread was considered to be a replicate for that sampling period and all byssal threads were assumed to be independent of one another. Initial strength and extensibility were analyzed as a fixed factor (season) one-way ANOVA ($P=0.05$). Regression

analyses were used to identify the impact of exposure on both breaking force and strain for each season ($P=0.05$). A general linear model was used to compare the regression slopes between seasons ($P=0.05$).

Material properties and attachment strength

Two mathematical models, described in Bell and Gosline (Bell and Gosline, 1996), were used to estimate mussel attachment strength from the initial material properties each season. One model estimates the attachment strength of a mussel when it is displaced parallel to the substrate simulating removal due to the hydrodynamic force of drag, while a second model provides estimates for perpendicular loading, simulating lift (Bell and Gosline, 1997; Denny, 1987). For each model, it was assumed that the byssal complex was composed of 50 radially arranged threads of a representative length for each season (see Results). Seasonal estimates of breaking force, breaking strain, yield force and stiffness were obtained from the tensile tests described above. In these models, initial stiffness, k_1 , was defined as the slope of the tangent to the initial portion of the force-extension curve (calculated as $F_y/\epsilon_y \times l_0$). Average stiffness, k_2 , is the average slope of the entire force-extension curve (calculated as $F_b/\epsilon_b \times l_0$). Average stiffness was calculated for only those seasons in which threads predominantly failed beyond the yield force.

Results

Spring threads were over 60% stronger, withstanding forces averaging 0.19 N, and over 83% more extensible, with a breaking strain of 0.64, than threads produced during the other seasons ($P<0.001$; Table 1). The ultimate properties of threads from summer, fall and winter were statistically indistinguishable (Table 1). In the majority of spring threads,

Table 1. Summary of thread mechanical properties for initial lab-produced threads (1–7 days old; never outplanted)

Season	Breaking properties		Yield properties	
	Force (N)	Strain (mm mm ⁻¹)	Force (N)	Strain (mm mm ⁻¹)
Spring	0.19±0.01 ^a	0.64±0.08 ^a	0.14±0.02	0.39±0.06
Summer	0.12±0.02 ^b	0.35±0.04 ^b	0.14±0.03	0.32±0.04
Fall	0.08±0.01 ^b	0.33±0.05 ^b	0.09±0.01	0.33±0.05
Winter	0.08±0.01 ^b	0.35±0.04 ^b	0.12±0.02	0.36±0.05
<i>P</i> -value	<0.001	<0.001	0.21	0.74

Breaking force and strain are for all initial threads ($N=15-18$), while yield force and strain are for only those lab-produced threads that extended past the yield phase of the force-extension curve ($N=7-10$). ANOVA analyses indicated that both breaking force and strain varied significantly with season; multiple comparison tests separated seasons into significantly different groups, as denoted by the superscripts. Tests were performed in 15°C seawater at an extension rate of 10 mm min⁻¹. All values are means \pm s.e.m.

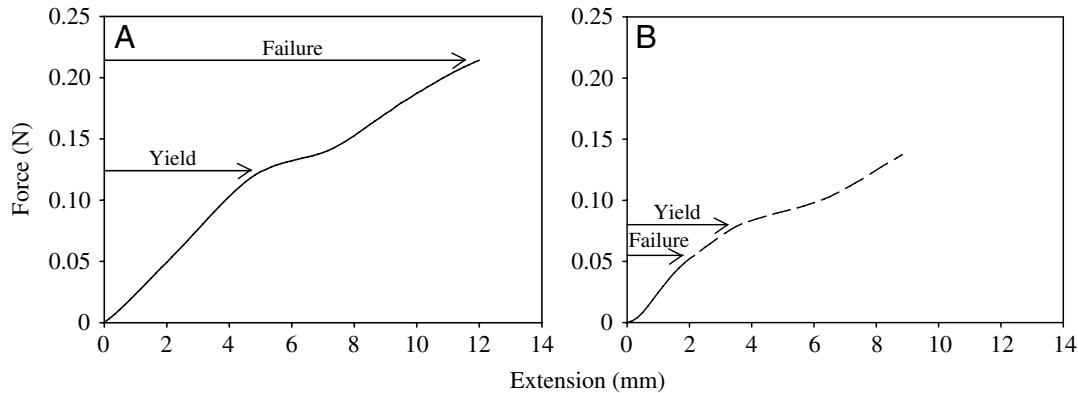


Fig. 1. Force–extension behavior of representative spring and fall threads. (A) The thread produced in spring extended beyond the yield point and stiffened again before failing at the plaque ($F_b=0.21$ N). (B) The thread produced in fall broke at the plaque during the first tensile test (solid line, $F_b=0.05$ N). When retested without the plaque (broken line), the thread extended beyond the yield point and stiffened before failing in the proximal region ($F_b=0.14$ N). Threads produced in both summer and winter were similar to fall threads, breaking before reaching the second (yield) phase of the force–extension curve (data not shown).

breaking force was higher than the observed yield force (Fig. 1A; Table 1). By contrast, threads from all other seasons usually failed at forces lower than the yield force (Fig. 1B; Table 1). In such cases, it was necessary to retest threads that initially failed at the plaque to identify the yield force from the force–extension curves (Table 1). Yield force and strain were not statistically distinguishable among seasons ($P=0.21$ and $P=0.74$, respectively; Table 1).

For each season, the majority of initial breaks occurred in the plaque region, with only plaque breaks during the winter experiment (Table 2). Failure in the proximal region was also common in the remaining seasons, while distal breaks were

relatively rare. The breaking force was not significantly different between plaque and proximal breaks for each season (t -tests; $P=0.13$ – 0.90). The strength of the plaque and proximal region varied with season ($P<0.001$ and $P<0.01$, respectively; Table 2), with significantly higher values recorded in spring.

Thread mechanical properties were influenced by the duration of exposure in some seasons but not others (Figs 2, 3; Table 3). Breaking strain decreased with exposure in all seasons except fall ($P<0.05$; Table 3); thread extensibility decreased 41–54% over four weeks of exposure (Fig. 3; Table 3). Breaking force also decreased with exposure time, but only in the spring and summer experiments ($P<0.05$; Fig. 2). The proximal portions of many threads were missing

Table 2. Summary of byssal thread breaking force with respect to break location (plaque, proximal and distal regions) in initial lab-produced threads (1–7 days old; never outplanted)

Season	Breaking force (N)		
	Plaque	Proximal	Distal
Spring	0.20±0.01 (8) ^a	0.18±0.02 (7) ^a	NA
Summer	0.09±0.02 (7) ^b	0.10±0.02 (5) ^b	0.19±0.05 (4)
Fall	0.07±0.01 (11) ^b	0.08±0.02 (5) ^b	0.12±0.00 (2)
Winter	0.08±0.01 (16) ^b	NA	NA
<i>P</i> -value	<0.001	<0.01	NA

For each season, the breaking force required for failure was not significantly different between plaque and proximal breaks. Tests were performed in 15°C seawater at an extension rate of 10 mm min⁻¹. All values are means ± s.e.m., with sample size listed in parentheses. *P*-value is for ANOVA analyses of thread mechanics among seasons; multiple comparison tests separated seasons into significantly different groups, as denoted by the superscripts. Distal region failures were rare (NA, not available) and were therefore omitted from ANOVA analysis.

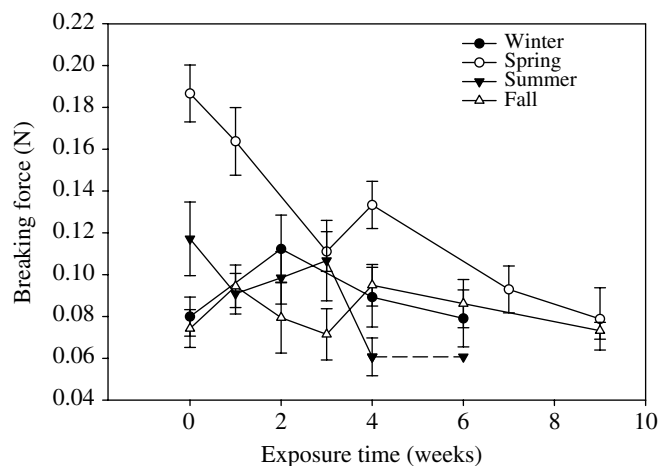


Fig. 2. Breaking force for threads over time of exposure in four seasons. Symbols represent means ± s.e.m. values ($N=11$ – 29). Tests were performed in 15°C seawater at an extension rate of 10 mm min⁻¹. Note that no testable threads persisted beyond four weeks during the summer; the value for six weeks exposure was conservatively estimated from the four-week value (broken line).

Table 3. Summary of the effect of exposure time on thread mechanical properties (breaking load and strain, estimated lifetime) among seasons

	Season			
	Spring	Summer	Fall	Winter
Breaking force				
<i>P</i> -value	<0.01	0.04	0.71	0.79
<i>r</i> ²	0.88	0.68	–	–
$\Delta F_b/\text{time}$	-0.011	-0.009	–	–
Decay (%)	29	48	–	–
Breaking strain				
<i>P</i> -value	<0.01	0.04	0.08	0.01
<i>r</i> ²	0.85	0.67	–	0.99
$\Delta F_b/\text{time}$	-0.047	-0.023	–	-0.032
Decay (%)	54	42	–	41
Thread lifetime (weeks)	9+	<6	9+	9+

Regression statistics (*r*²; slope, $\Delta F_b/\text{time}$ in weeks) and actual decay over four weeks (Decay) are presented only when the effect of exposure was significant. Thread lifetime was estimated from exposure experiments and was the period of time during which testable threads were present (Moeser, 2004). Seasonal regressions were not significantly different for either breaking force or breaking strain. For each material property, the slope of the regressions did not differ among seasons (force, *P*=0.19; strain, *P*=0.09).

towards the end of the exposure experiments and many threads were so fragile that they fell apart during preparation for tensile testing. These difficulties were magnified during the summer experiment; only one testable thread remained on the granite slabs after six weeks of exposure, and heavy biofouling was

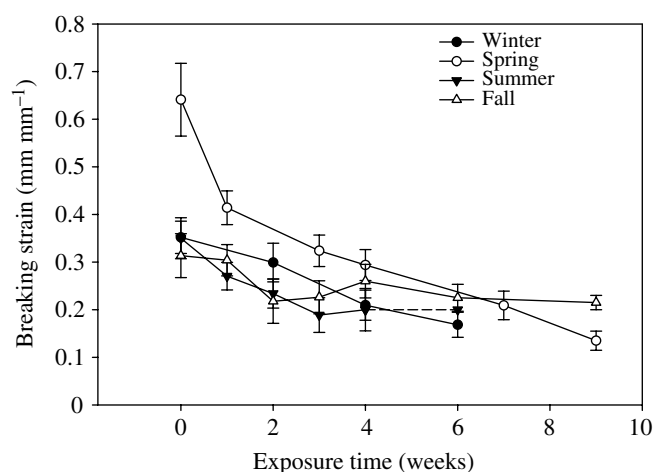


Fig. 3. Breaking strain for threads over time of exposure in four seasons. Symbols represent means \pm s.e.m. values (*N*=11–29). Tests were performed in a 15°C seawater bath at an extension rate of 10 mm min⁻¹. Note that no testable threads persisted beyond four weeks during the summer; the value for six weeks exposure was conservatively estimated from the four-week values (broken line).

Table 4. Summary of mussel condition across seasons

Season	Thread	Gonad index	Condition index
	length (mm)	(g gonad g ⁻¹ tissue $\times 10^{-2}$)	(g mm ⁻³ $\times 10^{-6}$)
Spring	13.45 \pm 1.16 ^{a,b}	11.42 \pm 0.32 ^b	4.39 \pm 0.06 ^a
Summer	16.82 \pm 1.49 ^a	18.01 \pm 0.63 ^a	4.41 \pm 0.08 ^a
Fall	11.21 \pm 0.55 ^b	9.53 \pm 0.25 ^b	2.29 \pm 0.06 ^b
Winter	10.14 \pm 0.49 ^b	10.33 \pm 0.20 ^b	3.91 \pm 0.05 ^a
<i>P</i> -value	<0.001	<0.001	<0.001

Thread length values are for lab-produced threads 1–7 days old (never outplanted; *N*=15–18). Gonad index and condition index values are for all mussels collected at the onset of each experiment (*N*=45–50). ANOVA analyses indicated that all mussel properties varied significantly with season; multiple comparison tests separated seasons into significantly different groups, as denoted by the superscripts. All values are means \pm s.e.m.

observed (Fig. 4). To enable summer regression analyses, we conservatively estimated six-week values as the mean of four-week samples; true values are likely much lower.

Thread length and mussel condition also varied seasonally. Summer threads were significantly longer than fall and winter threads, but were statistically indistinguishable from spring threads (Table 4). Summer mussels also exhibited significantly higher gonad indices in comparison with all other seasons, which were statistically indistinguishable (Table 4). Mean condition index of fall mussels was significantly lower in comparison with all other seasons (Table 4).

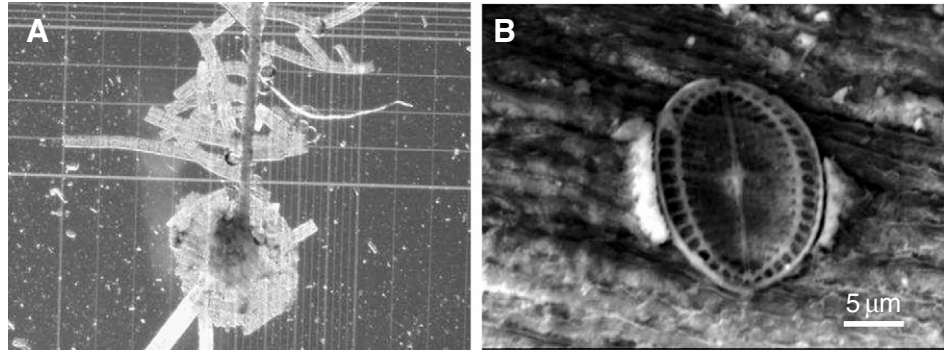
The models predict lower tenacity when mussels are displaced parallel to the substrate for all seasons (Table 5). For either displacement direction, the weakest attachment is predicted to occur during the fall when threads are weak, inextensible and short (Table 5). Attachment in spring is predicted to be over twofold stronger than fall, regardless of

Table 5. Input parameters and results for the models estimating mussel attachment strength for all seasons, with either perpendicular or parallel displacement

	Season			
	Spring	Summer	Fall	Winter
Input parameters				
Initial stiffness, <i>k</i> ₁ (N m ⁻¹)	28.0	26.8	26.3	31.4
Average stiffness, <i>k</i> ₂ (N m ⁻¹)	21.6			
Model results				
Perpendicular attachment force (N)	8.50	4.31	2.79	2.98
Parallel attachment force (N)	3.56	2.64	1.64	1.98

Mussels were assumed to have 50 radially arranged threads that varied in length and material properties according to the seasonal averages listed in Table 1. Average stiffness was calculated for only those seasons that failed beyond the yield force. See Bell and Gosline (Bell and Gosline, 1996) for a detailed description of the models.

Fig. 4. Biofouling of byssal threads exposed to field conditions for three weeks during the summer decay experiment. (A) Compound microscope photograph of distal region and adhesive plaque of a byssal thread (at 16 \times magnification). Note the extensive biofouling by chain-forming diatoms. (B) SEM image of distal portion of a byssal thread in which a diatom is partially embedded. Image provided by Shanna Brazee.



displacement direction, while summer and winter mussel attachment is intermediate (Table 5). Absolute seasonal differences in attachment strength are magnified with perpendicular loading due to the higher overall predictions. These general trends in predicted attachment strength remained when the input of thread length was not varied seasonally (data not shown).

Discussion

This study demonstrates that both thread quality and rates of decay vary with season and that these factors are likely to significantly influence mussel attachment strength. Threads produced during the spring are 1.6–2.4 times stronger and nearly twice as extensible in comparison with other seasons. Based on numerical modeling, these material properties combine to enhance mussel attachment strength in spring two- to threefold compared with other seasons. Thus, seasonal variation in thread mechanics can produce an annual cycle in attachment strength that is consistent with observed patterns in Rhode Island mussel populations (Carrington, 2002).

The high strength of threads produced in spring is directly attributable to the relatively high strength of both the plaque and proximal region, the two most common locations of failure. As was observed by Bell and Gosline (Bell and Gosline, 1996), these two locations were closely matched in strength within each season, suggesting that either region serves as the weak link in each ‘chain’ (thread) that anchors a mussel to its substrate. Even a subtle difference in the strength of these two regions is sufficient to bias failure exclusively to one region, as was observed in winter.

Importantly, the reduction of the plaque and proximal region strength in summer through winter not only reduces thread strength but also reduces thread extensibility. In these seasons, the majority of threads tested broke before reaching the yield point. As a consequence, the large deformation (yield) in the distal region was not realized and overall thread extensibility was greatly reduced. By contrast, the plaques and proximal regions of spring threads were strong enough to break well beyond the yield point, and the classic triphasic force–extension behavior of whole threads described by Bell and Gosline (Bell and Gosline, 1996) was consistently observed in this season only. Because thread extensibility

allows for thread realignment and recruitment, the twofold increase in thread extensibility in spring greatly contributes to the higher attachment strength of mussels in spring compared with other seasons. This analysis highlights the importance of examining the mechanical behavior of each region of the thread, not only the region(s) of failure; estimates of attachment strength based on thread strength alone would be gross underestimates.

While the mechanism for the observed differences in initial thread strength and extensibility are unknown, numerous environmental and physiological factors may be involved. For example, the presence of certain metal ions are not only necessary for adhesion (Monahan and Wilker, 2004), but iron and manganese in particular are necessary for the cross-linking of byssal thread proteins (Sever et al., 2004; Waite et al., 2002); metal ions are absorbed by the mussel through active filtration while feeding and are then used to crosslink the proteins within the thread (Sun and Waite, 2005). It is unknown whether the presence of these elements in seawater varies seasonally or how they affect thread quality. Additionally, inferior thread quality coincides with low mussel condition and elevated water temperature. It is possible that lower food availability alters thread composition or that temperature affects the molding and curing process during byssal thread formation.

Attachment strength not only reflects the initial material properties of threads but also the rate at which these threads decay. Price found that threads beyond 19 days in age broke less cleanly and exhibited reduced stress and a reduced Young’s modulus in comparison with younger threads (Price, 1981). In the present study, thread extensibility decreased more than 40% after four weeks of exposure in all seasons except fall, while breaking force decreased over 29% for spring and summer (Table 3). These results indicate that exposure significantly influences the material properties of byssal threads even during the seasons with strong attachment. It is notable, however, that spring threads are initially superior and need to be exposed for over four weeks before mechanical properties degrade to the initial level of the fall threads (Figs 2, 3).

Thread decay could play an even more influential role than presented here, as the decay rates reported in this study are most likely underestimates. Threads were only included in the analyses if they were whole, able to withstand the manipulation

necessary to connect them to the tensometer and did not break at the grips. These requirements severely limited the number of threads that could be included in this study, especially during summer, thus underestimating the rate of decay. Nonetheless, thread life spans were found to be greater than nine weeks for all seasons except summer. This extended lifespan could potentially increase attachment strength both within a season and within the subsequent season by providing additional threads with which to withstand hydrodynamic forces. For example, due to the short lifespan of summer threads from increased biofouling, fall mussels have a reduced 'baseline' of attachment. Thread production rate also increases in summer (Moeser et al., 2006), which may ameliorate the negative effects of thread decay in this season.

In this study, a larger portion of breaks occurred at the plaque–substrate interface, even though there was no significant difference in breaking force between plaque and proximal breaks. Threads were extended perpendicularly from the substrate in this experiment, thereby possibly providing them with more opportunity to 'peel away' from the substrate than is found in natural environments. If threads were extended at an acute angle to the substrate, proximal breaks could become more common. Regardless of this potential plaque failure bias, the general conclusions of this study would not be affected.

Seasonal variations in mussel attachment strength have been observed both in Rhode Island, USA and England, UK (Carrington, 2002; Price, 1980; Price, 1982). Peak attachment strength in Rhode Island occurs in late winter and early spring, following hurricane season and preceding the period of increased gamete production. This seasonal pattern prompted Carrington to suggest an energetic trade-off between gamete production and attachment, with thread production increasing only after spawning periods (Carrington, 2002). Moeser and

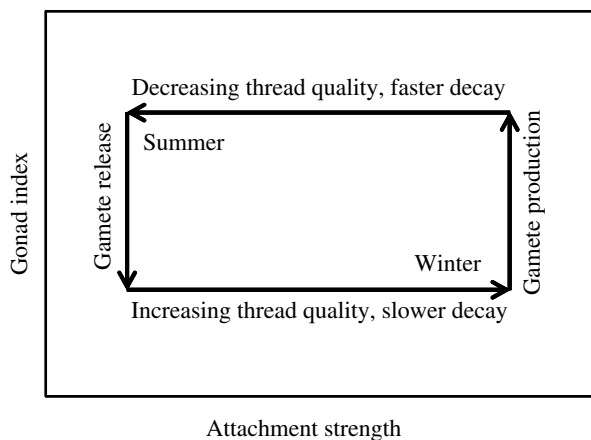


Fig. 5. A modified version of the scheme proposed by Carrington (Carrington, 2002) to explain the seasonal variation observed in tenacity, or attachment strength. While the originally proposed energetic trade-off between gamete production and tenacity is maintained, it is now proposed that an annual cycle of tenacity is due to variation in thread quality, not quantity.

coworkers, however, do not support thread production as a likely mechanism for increased mussel attachment (Moeser et al., 2006). As an alternative, we suggest that the energetic trade-off manifests itself in the quality, not quantity, of threads produced seasonally. Thread strength and extensibility increase after fall and winter, leading to the strongest attachment during the spring, at which point energetic resources switch their focus towards gamete production (Fig. 5). This shift in energetic allocation, combined with an increased rate of decay, decreases attachment strength throughout the summer, leading to the weakest attachment strength in the fall (Fig. 5). The seasonal pattern of mussel condition and thread mechanical properties in this study are consistent with this modified scheme.

Overall, this study indicates that material properties strongly influence the dynamics of attachment strength of *M. edulis*. Although the literature has focused on the integration of wave action and thread production as the primary process affecting attachment strength, the present study suggests that seasonal variations in material properties play an even more significant role in determining mussel attachment strength. Ultimately, it is the combination of thread mechanics, decay and production that influence overall attachment strength. Future studies are needed to clarify the interactions between these factors and to elucidate how environmental and physiological conditions contribute to seasonal byssal thread mechanics.

List of abbreviations and symbols

GI	gonad index
CI	condition index
F_b	breaking force
ϵ_b	breaking strain
F_y	yield force
ϵ_y	yield strain
l_0	thread length
k_1	initial stiffness
k_2	average stiffness

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