2006

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Citation/Publisher Attribution

Moeser, G. M., Leba, H., & Carrington, E. (2006). Seasonal influence of wave action on thread production in *Mytilus edulis*. *Journal of Experimental Biology, 209*, 881-890. doi: 10.1242/jeb.02050
Available at: https://doi.org/10.1242/jeb.02050

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The Journal of Experimental Biology 209, 881-890
Published by The Company of Biologists 2006
doi:10.1242/jeb.02050

Seasonal influence of wave action on thread production in *Mytilus edulis*

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Accepted 20 December 2005

Summary

The blue mussel *Mytilus edulis* maintains a strong attachment to the substrate in high energy environments by producing byssal threads. On the shores of Rhode Island, USA, mussel attachment strength increases twofold in spring compared to that in the fall. While many factors could influence attachment strength (temperature, food supply, predator cues, etc.), it has been proposed that the variation observed is primarily due to increased thread production during winter and spring in response to increased wave action. This study evaluates the influence of three aspects of wave action on the thread production of *M. edulis*. Mussels were exposed to flow, acceleration and byssal loading stimuli and the subsequent number of byssal threads produced in the laboratory was monitored. Increased flow elicited the strongest response, significantly decreasing thread production in mussels. This result was confirmed in flume experiments exposing mussels to a range of flows, with reduced thread production above 15 cm s^{-1}. The influence of both acceleration and byssal loading was sporadic and inconsistent across seasons. Surprisingly, overall thread production in the laboratory was lowest in winter, a time when mussels typically peak in attachment. A similar seasonal pattern was observed in field assays, with high thread production during periods of elevated temperature, reduced wave action, and high reproductive condition. These results suggest that seasonal variation in attachment strength does not reflect increased thread production in response to wave action, and that other possible factors, such as seasonal variability in both the material properties of byssal threads and thread decay rates, warrant further investigation.

Key words: mussel, *Mytilus edulis*, byssal thread, flow, temperature.

Introduction

The rocky intertidal zone is an extremely dynamic environment in which sessile organisms rely on their attachment to the substrate for survival. Mussels are dominant competitors for space in this environment, in part because of their ability to maintain a strong attachment (Bell and Gosline, 1996; Carrington and Gosline, 2004). Mussels form dense assemblages that not only help secure their own survival, but also buffer other organisms from the physical stresses of the intertidal zone (Bertness and Leonard, 1997). As dominant competitors and important bioengineers, mussels exhibit a strong influence on the diversity and structure of the intertidal community (Paine, 1974; Suchanek, 1978).

Mytilus edulis Linnaeus, the blue mussel, tethers itself to the substrate by producing a byssal complex composed of multiple extracellular collagenous byssal threads. Each thread is secreted by a gland in the foot in a manner similar to polymer injection molding, and terminates at an adhesive plaque which attaches the thread to the substrate (Waite, 1992). By varying the number of threads produced, *M. edulis* may be able to influence the strength of its attachment (Bell and Gosline, 1997; Carrington, 2002a). Along with thread number, variations in the mechanical quality and the rate at which threads decay may also influence mussel attachment to the substrate (Bell and Gosline, 1996; Moeser, 2004).

Flows produced by large breaking waves generate hydrodynamic forces (drag and lift) on organisms in the intertidal zone (Bell and Gosline, 1997; Denny, 1987; Denny, 1988; Witman and Suchanek, 1984). A mussel will be dislodged from the substrate when these forces exceed its attachment strength (Carrington, 2002a; Carrington, 2002b; Denny et al., 1985). Storms represent one of the largest survival risks for intertidal mussels because these events increase wave action over a period of 1–2 days and can quickly dislodge individuals with weak attachment. In the North Atlantic, extreme storms such as hurricanes have increased in frequency and severity since 1995 (Goldenberg et al., 2001). Mean wave height has also increased by 2% per year over the latter half of the 20th century (Bacon and Carter, 1991; Hoozemans and Wiersma, 1992). If these trends continue, hydrodynamic forces within the intertidal zone are predicted to increase concomitantly, thereby increasing dislodgement rates, unless mussels can increase their attachment strength (Carrington, 2002a; Helmuth et al., 2005).

A twofold annual variation in attachment strength, or tenacity, has been observed for *M. edulis* on both Rhode Island, USA and British rocky shores (Carrington, 2002a; Price, 1980; Price, 1982). Price (Price, 1982) found that peak mussel attachment preceded storm seasons, suggesting that mussels anticipated increases in wave action by increasing attachment strength. However, Carrington (Carrington, 2002a) observed a different pattern on Rhode Island shores, with attachment strength peaking during late winter and early spring. In this case, increased attachment strength followed a period of increased wave action caused by hurricanes and winter storms and coincided with reduced reproductive effort and water temperature (Carrington, 2002a). This most probably reflects a strengthening of the entire population of mussels rather than a distributional shift due to fall-off of weak mussels (Carrington, 2002a). Since this increase in attachment strength occurs at a different time in the East Atlantic compared to the West Atlantic, it is difficult to determine which, if any, environmental or physiological factors influence this variation.

'Wave action' is a qualitative term that refers to small-scale turbulence superimposed on a directional current that is created by waves breaking on the shore (Bell and Denny, 1994; Denny, 1988). Intense wave action creates extreme hydrodynamic forces which, in turn, increase the risk of mussel dislodgment (Bell and Gosline, 1997; Carrington, 2002a; Denny, 1987; Hunt and Scheibling, 2001). Wave action exposes mussels to three potential stimuli for increased thread production: (1) mean flow, (2) acceleration (vertical displacement of the mussel body) and (3) hydrodynamic loading of the byssal retractor muscle by transferring tension from the byssal complex. Of these potential stimuli, flow is presently thought to be the primary cue for increased thread production in *M. edulis* (Dolmer and Svane, 1994; Lee et al., 1990; Van Winkle, 1970; Witman and Suchanek, 1984; Young, 1985). Previous research has suggested a positive linear relationship between water flow and mussel attachment (Dolmer and Svane, 1994). However, mussels held firmly against the substrate exhibit poor thread production, even under high water velocities (Seed and Suchanek, 1992); suggesting that flow is not the only factor influencing thread production. The response of mussels to agitation, the combination of both acceleration and byssal loading, has been examined to a lesser extent, with conflicting results. Van Winkle (Van Winkle, 1970) determined that increased agitation of *Geukensia demissa* led to a 33% decrease in thread production when compared with stationary mussels, whereas Young (Young 1985) suggested that thread production increases notably with increased rate of agitation in *M. edulis*. Thus it is unclear how agitation, and more specifically acceleration and byssal loading, affect thread production and attachment strength in *M. edulis*.

The purpose of this study is to identify whether wave action influences thread production thereby driving the temporal variation in the attachment strength of *M. edulis* on Rhode Island shores. Three aspects of wave action (flow, acceleration and byssal loading) were simulated in the laboratory to determine which stimulus, or combination thereof, was most important in cueing thread production. Flume experiments were also performed to detail the relationship between thread production and flow, and thread production was monitored in the field, seasonally. Together, these experiments indicate that mussels do not respond to wave action with increased thread production and that other explanations for seasonally variable attachment strength warrant further investigation.

Materials and methods

This study comprises three experiments, two conducted in the laboratory and one in the field, which are described in more detail below. For both the field and flume experiments, *Mytilus edulis* L. of similar length (approximately 4 cm) were collected from the rocky intertidal zone at either Bass Rock (41.4°N, 71.5°W; for site description, see Carrington 2002a) or Black Point (1 km south of Bass Rock) in Rhode Island Sound, USA. For the simulated wave action experiment, mussels were collected from the locations listed above, however, length varied from 30–50 cm in the two factor experiments; length was used as a covariate in applicable statistical analyses. Mussels were held in laboratory aquaria at ambient temperature for 1–4 days prior to experimentation; only mussels that produced greater than three threads during this acclimation period were chosen as subjects.

Because mussel attachment strength may depend on mussel condition (Carrington, 2002a), 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 then the mantle, including the gonads, was separated from the remaining body tissue for each mussel. The mantle and the remaining body tissue were then dried to a constant weight at 60°C (1–2 days). GI was calculated as the dry mantle mass divided by the total dry tissue mass (Carrington, 2002a). CI was calculated as the total dry tissue mass divided by the shell length cubed (Baird, 1958), where shell length cubed was used as a proxy for volume (Bell and Gosline, 1997).

Wave and water temperature conditions were monitored at Bass Rock throughout the study. A SBE26 Seaguage (Seabird Electronics, Bellevue, WA, USA) was mounted at a depth of 7 m approximately 200 m offshore. The sensor recorded temperature every 15 min and burst measurements of pressure (1024 points at 2 Hz) every 4 h. The pressure data were processed with Seasoft software (Seabird Electronics, Bellevue, WA, USA) to obtain significant wave height (H_s) of each burst. Daily averages of water temperature and H_s were calculated for comparison with experimental data.

Statistical analyses for the simulated wave action experiments were performed using SAS 9.0 statistical software (Cary, NC, USA); all other data were analyzed using SigmaStat 3.1 (Systat; Richmond, CA, USA).

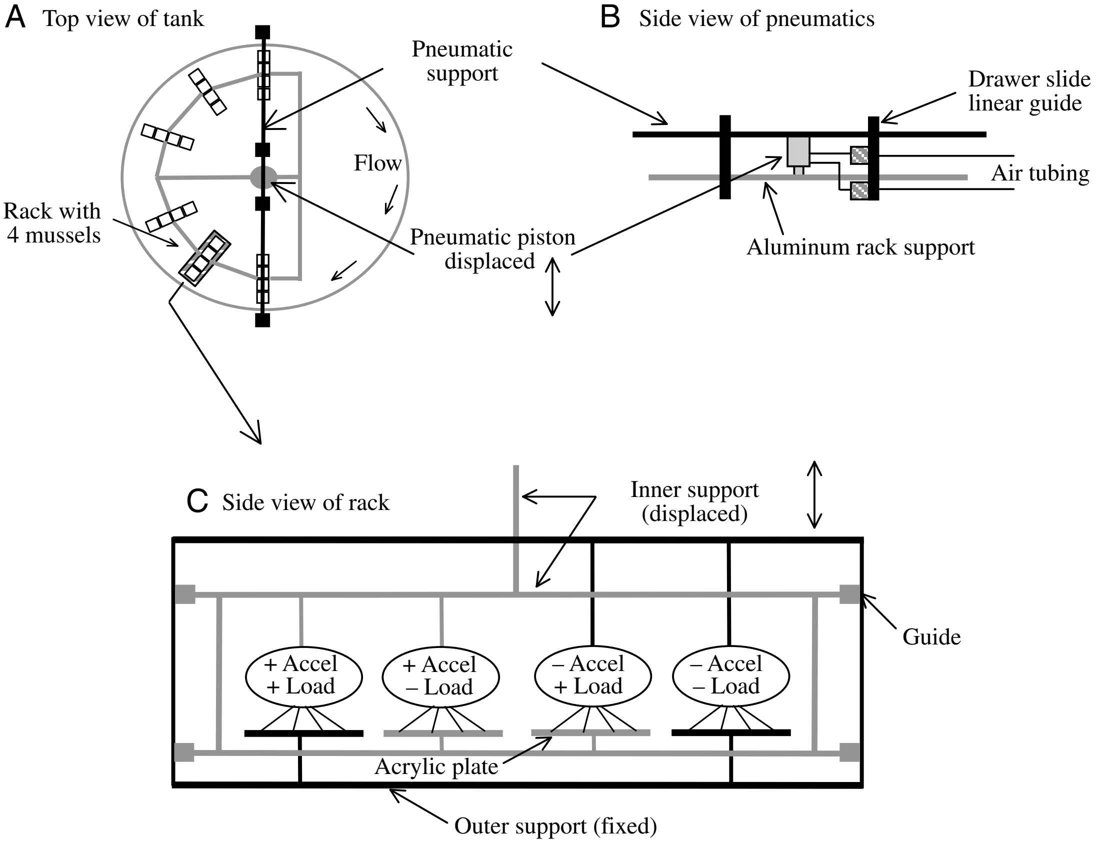

Fig. 1. Schematic of the aquarium set-up for the laboratory experiments. (A) Top view of replicate tank. Six racks were attached to an aluminum semi-circle connected to a central pneumatic piston. A single flow level, high or low, was established in each tank by circulating the water with a trawling motor. Each rack was placed on the periphery of the tank to minimize flow gradients. (B) Side view of pneumatics. Drawer-slides were used as guides to maintain a linear displacement of the aluminum rack support and the individual racks. These slides were attached both to the rack support and the pneumatic support crossbeam. The pneumatic piston was attached to the aluminum rack support and displaced ±2.5 mm. The piston was forced to change directions when an upper or lower pneumatic button was activated. Air lines connected the buttons to the pistons and back to the regulator. (C) Side view of a single rack. Each rack supported four mussels, each with a different combination of acceleration and byssal loading. The inner support was attached to the aluminum semi-circle and vertically displaced ±2.5 mm. The outer support was attached to the bottom of the tank and held fixed throughout the experiment. The order of each treatment was randomized between racks. Accel, acceleration; Load, byssal load.

Simulated wave action experiment

Laboratory experiments were designed to evaluate the effect of flow, acceleration and byssal loading on thread production and were repeated seasonally during 2003–2004. Two large aquaria (60 cm deep, 152 cm diameter) were equipped with a pneumatic pump system used to create acceleration and byssal loading (Fig. 1). The water in each aquarium was pre-filtered to remove all particles >300 μm and held at a constant temperature that was adjusted with season (within 8°C of ambient water temperature; Moeser, 2004). Mussels were fed Post-Set 1800 Shellfish Diet (Reed Mariculture, Campbell, CA, USA) 5 days per week. An algal biomass equivalent to approximately two percent of the average dry mussel body mass per mussel per day was distributed to the mussels using a slow release drip feeder. The experimental water velocities were below the level where the filtering ability of mussels is severely impeded (Newell et al., 2001).

Each pneumatic pump was equipped with six racks, each containing four mussels and every combination of two components of wave action: acceleration and byssal loading (Fig. 1C). The right valve of each mussel was fixed to a vertical nylon rod using cyanoacrylate glue, which was then suspended 6.5 mm above an acrylic plate (Fig. 2). Preliminary experiments indicated that the suspension of mussels above the substrate did not significantly hinder thread production (G.M.M., unpublished data; H.L., unpublished data). Mussels were aligned with anterior ends downstream to remove any effects of orientation and each was randomly assigned to a three-factor treatment.

A total of eight three-factor treatments, consisting of every combination of (1) high or low flow, (2) presence or absence of acceleration, and (3) presence or absence of byssal loading, were established as follows.

(1) Flow

Unidirectional flow was established at approximately 8 cm s^{-1} (low flow) and 20 cm s^{-1} (high flow) in two tanks by

Fig. 2. One valve of each mussel was fixed to a vertical nylon rod using cyanoacrylate glue, which was then suspended 6.5 mm above an acrylic plate. During each experiment, mussels gradually tethered themselves to the acrylic plate by producing byssal threads.

circulating water with a transom-mount motor (Minn Kota Endura 40, Sidney, NE, USA; Fig. 1A). Water velocity was measured at mussel height between each rack using a Marsh McBirney 511 flowmeter (Frederick, MD, USA). Although the high flow treatment is well below velocities reported in the field (Hunt and Scheibling, 2001), preliminary studies indicated that velocities greater than 20 cm s^{-1} reduced thread production to impractical levels (G.M.M., unpublished data).

(2) Acceleration

The vertical rod attached to the mussel was connected to an inner support attached to the aluminum rack support which was oscillated ±2.5 mm at a frequency of 1.25 Hz by the pneumatic pump system (Fig. 1B,C), generating a maximum acceleration of approximately 0.10 m s^{-2}. Although the magnitude and frequency of this acceleration is lower than field measurements (Gaylord, 1999), preliminary trails determined this displacement and frequency maximized the response without hindering the mussels' ability to contact the substrate (G.M.M., unpublished). For those mussels experiencing no acceleration, the vertical rod was attached to a fixed support (Fig. 1C).

(3) Byssal loading

The distance between the mussel and the acrylic plate was oscillated ±2.5 mm by displacing either the plate or the mussel. This placed a fluctuating, tensile load of approximately 0.15 N on the mussel byssus, a load well within the range expected in the field (Bell and Gosline, 1996). The control treatment for this experiment consisted of low flow and the absence of both acceleration and byssal loading. A second set of control mussels confirmed that mussels attached to the racks were not affected by the motion of neighboring treatments (Moeser, 2004). For each treatment, mussels were stimulated continuously for 48 h to maximize mussel response (Moeser, 2004). The number of byssal plaques attached to each acrylic plate was then counted, byssal threads were cut and removed, and mussels were rotated to another treatment. A total of 48 mussels were rotated through each of the eight treatments over the 16-day experiment. Each mussel was considered a replicate for each treatment and independent from other mussels. This experiment was performed in two tanks and relied on pseudoreplication to create two flow regimes; separate controls failed to identify any tank effects on the ability of mussels to produce threads (Moeser, 2004).

Mussel GI and CI were estimated, as described above, at the close of each experiment. Neither of these indices significantly decreased during the experiments compared to controls; in preliminary analyses of the effect of GI, CI and mussel length on thread production, only the latter was found to be significant (Moeser, 2004).

The three factor, full factorial experiment was performed in August, February and October. Data were analyzed separately for each month as a univariate (number of threads produced), fixed factor (flow, acceleration and byssal loading), repeated measures three-way ANOVA with a full factorial design (P=0.05). Data were also analyzed as a univariate (number of threads), mixed factor (month, flow, acceleration and byssal loading) four-way ANOVA with a full factorial design (P=0.05). Owing to the difficulty of maintaining multiple replicate treatments, six additional experiments examined byssal loading and acceleration only. These two-factor experiments were performed at low flow (approximately 8 cm s^{-1}) and mussels were handled as described above (N=24). Data were analyzed as a univariate (number of threads produced), fixed factor (acceleration and byssal loading), repeated measures two-way ANOVA with a factorial design using mussel length as a covariate.

Seasonal thread production data were compared to field measurements of tenacity at Bass Rock. The latter were obtained from monthly measurements of intertidal mussels, averaged over a 6-year period, 1998–2003 (data from Carrington, 2002a; E.C., unpublished data). Regression analysis was used to evaluate the influence of seasonal thread production on tenacity.

Flume experiment

A flume study was conducted to evaluate the thread production response of mussels to a range of unidirectional water velocities, 5–20 cm s^{-1}. In July 2004, a circulating flow tank (Grace, 2004) was used to expose solitary mussels to a constant water velocity while byssal thread production was monitored over a 24-hour period. Each of ten mussels was mounted on nylon rods as described above. Each rod was attached to a rack that was placed flush with the floor of the working section of the flow tank (120 cm×17 cm×17 cm; L×W×H). Mussels were suspended 6.5 mm above the substrate, anterior facing downstream and separated by at least one shell length. Threads produced by each mussel were cut and counted every 24 h and the mussels were returned to the flume for another velocity trial. A total of ten trials were run at seven velocities; the velocity order was randomized among trials and mussels were replaced after 5 days. Seawater in the flume was aerated at 19°C and changed daily. A time-lapse video camera was used to observe mussel gaping and foot extension behavior. Nonlinear regression analysis was used to evaluate the effects of water velocity on mean byssal thread production.

Field experiment

Byssal thread production by mussels exposed to field conditions was quantified with a short term (4–5 day) assay repeated during nine spring low tides from October 2003 to July 2004. The byssus was gently cut from each of twenty mussels and shells were cleaned of byssal attachments. Each mussel was loosely tethered to an acrylic plate (5.3 mm thick; approximately 25 cm square) using a short length of braided nylon line (20 kg test). The line was affixed to each valve of the mussel using cyanoacrylate glue and further secured with marine epoxy (Devcon, Riviera Beach, FL, USA). The braided line was then threaded through pre-drilled holes ~2 cm apart and tied off on the underside of the acrylic plate. A temporary 5.3 mm spacer was used to ensure uniform slack in each tether. Four mussels were mounted on a total of five replicate plates; mussels were spaced a minimum of 4 cm apart with their anterior-posterior axis parallel to the plate.

Screws and plastic wall anchors were used to secure the plates with the attached mussels to a 1 m^2 horizontal area in the middle intertidal mussel zone at Bass Rock. Thread production was quantified daily by counting the adhesive plaques on the transparent plate and on the mussels themselves. Thread production rate (per day) was calculated as total threads divided by the assay duration. After 4–5 days, each mussel was dissected and weighed to determine the GI and CI as described above.

Differences in thread production rate among assay dates were evaluated using an ANOVA on square-root transformed data. A forward stepwise regression was used to predict mean thread production from the following mussel condition and environmental parameters: GI, CI, water temperature and H_s. Mussel condition indices were average values for each assay; environmental parameters were average daily mean values recorded during the assay. The criterion for parameter entry was $P<0.05$ for an F-test of the hypothesis that the parameter coefficient was zero.

Results

Simulated wave action

In the three factor experiments, flow elicited the greatest thread production response ($P<0.001$; Table 1). Surprisingly, increased flow consistently reduced thread production, although the magnitude of this effect depended on the month ($P<0.001$; Fig. 3). For example, high flow decreased thread production by only 18% in February, and by 50% in August and October (Fig. 3). Independent of season, only flow and byssal loading had significant effects on thread production; mussels produced half as many threads under high flow and 15% fewer threads when subjected to byssal loading ($P<0.001$ and $P<0.01$; Table 1; Fig. 4). This negative response to byssal loading varied with level of flow, such that thread production was most impaired under high flow conditions (flow×load interaction; $P=0.05$; Table 1). The interaction of acceleration and byssal loading varied with month (month×acceleration×load interaction; $P=0.02$; Table 1). In August, thread production increased when exposed to byssal loading in the absence of acceleration and decreased in the presence of both byssal loading and acceleration; in October and February thread production decreased when exposed to byssal loading with or without acceleration (Fig. 3).

Table 1. *Summary of the full factorial ANOVA analysis of the effect of three factors of wave action and month on thread production over a 48 h period in Mytilus edulis*

Source	Numerator d.f.	Denominator d.f.	F	P-value
Month	2	123	9.36	$<0.01^{\dagger}$
Flow	1	123	130.25	$<0.001^{\dagger}$
Accel	1	128	2.53	0.11
Load	1	128	11.62	$<0.01^{\dagger}$
Month×Flow	2	123	9.68	$<0.001^{\dagger}$
Month×Accel	2	128	1.64	0.19
Month×Load	2	128	1.32	0.27
Flow×Accel	1	478	1.03	0.31
Flow×Load	1	478	3.75	$0.05^{\dagger}$
Accel×Load	1	478	0.10	0.75
Month×Flow×Accel	2	478	2.25	0.10
Month×Flow×Load	2	478	0.61	0.54
Month×Accel×Load	2	478	3.73	$0.02^{\dagger}$
Flow×Accel×Load	1	478	0.08	0.77
Month×Flow×Accel ×Load	2	478	2.65	0.07

Owing to the large number of factors, this analysis was performed in SAS using the Proc Mixed procedure where threads produced (per 48 h) was the dependant variable and the independent variables were month in which the experiment was performed (Month), level of flow (Flow), and presence or absence of acceleration (Accel) and byssal loading (Load). Instead of the sum of squares, this procedure provides the degrees of freedom (d.f.) for each test performed in the model. $^{\dagger}P<0.05$.

In the remaining experiments, conducted under low flow conditions, acceleration and byssal loading had only a minor influence on thread production (Table 2). Specifically, the effects of these two factors were significant only in early June ($P<0.01$, byssal loading) and late June ($P<0.01$, acceleration×load interaction; Fig. 5). In contrast, all other experiments failed to demonstrate a significant effect of either acceleration or byssal loading on thread production. When the data are pooled by month, a clear seasonal pattern is observed with elevated thread production in summer. This pattern roughly corresponds to annual cycles in water temperature, GI and CI (Fig. 6). However, none of these potential factors were significantly correlated with thread production in the laboratory ($P=0.06$–0.58).

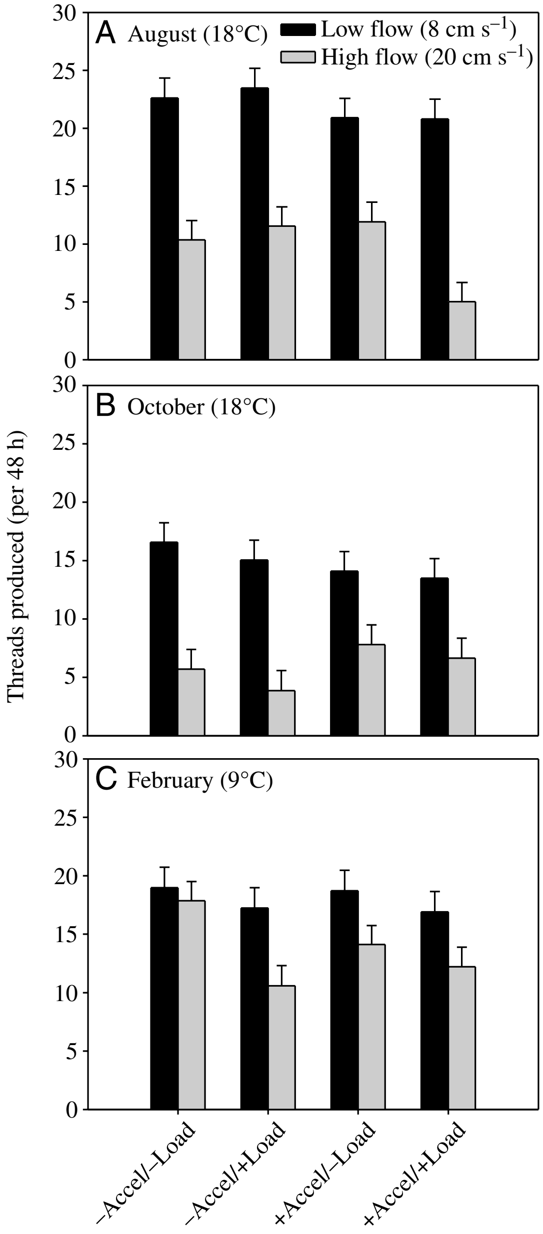

Fig. 3. Average number of threads produced over a 48 h period in the three factor experiments. Bars represent the least square mean (± s.e.m.) of threads produced for each treatment. The temperature at which each experiment was performed is indicated in parentheses. (A) August. (B) October. (C) February. Accel, acceleration.

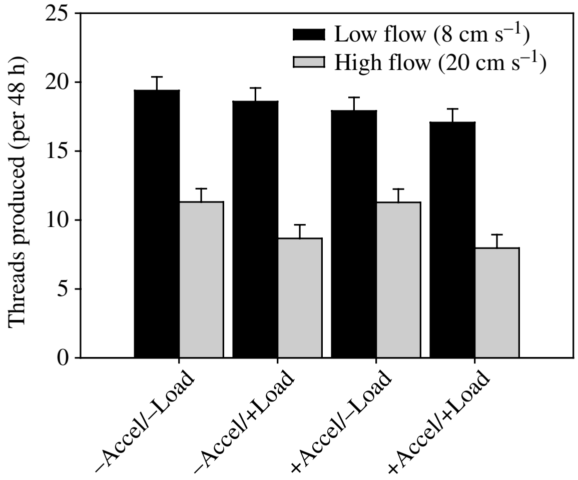

Fig. 4. Number of threads produced over a 48 h period for mussels experiencing each of eight combinations of flow, acceleration (Accel) and byssal loading (Load). Data is pooled for the three factor experiments (August, October and February). Bars represent the least square mean (± s.e.m.) of threads produced over 48 h (N=131). High flow and byssal loading significantly reduce thread production.

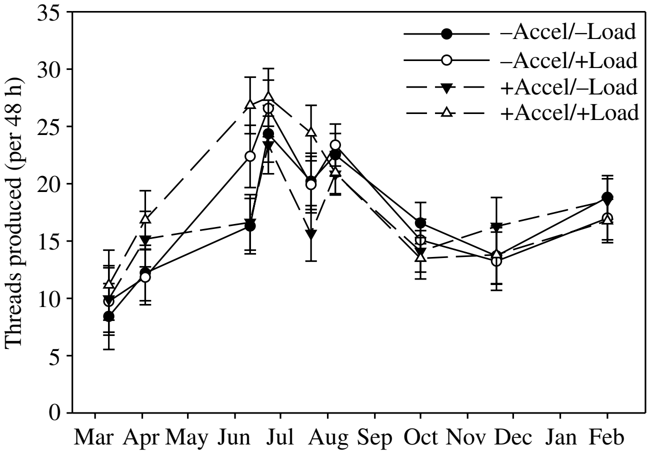

Fig. 5. Number of threads produced over a 48 h period for each combination of acceleration (Accel) and byssal loading (Load). Flow was kept constant at 8 cm s^{-1} (low flow treatment). Symbols represent the least square mean (± s.e.m.) of threads produced for each experiment. Each experiment was performed in the laboratory within 4–8°C of water temperature in the field.

Flume experiment

Byssal thread production by mussels exposed to constant flow varied nonlinearly as a function of water velocity (Fig. 7). A second order polynomial regression fit the data well (r^2=0.96), peaking at 10.6 cm s^{-1}. The first and second order coefficients were both significant (P<0.05), indicating a curvilinear relationship. Foot extension, which is necessary for thread formation, was visibly hindered at velocities >18 cm s^{-1}; high flow dragged the foot downstream and caused premature foot retraction.

Field experiment

Mussels produced threads steadily during each 4–5 day deployment, but this rate of production (total threads per day) varied significantly among assay dates (Table 3; P<0.001). Specifically, thread production was significantly lower in February and March, with the rate three to four times higher in all other months. Three independent variables were entered into the stepwise multiple regression analysis of thread production (Table 4); CI was not entered because of colinearity with GI. Water temperature explained the greatest

Table 2. *Summary of ANOVA analyses on the effects of acceleration and byssal loading on thread production over a 48 h period in Mytilus edulis*

Experiment	Source	d.f.	Type III SS	Mean square	*F*	*P*-value
March	Accel	1	28.85	28.85	0.59	0.45
	Load	1	34.30	34.30	0.47	0.50
	Accel×Load	1	0.02	0.02	<0.01	0.98
April	Accel	1	292.83	292.83	2.70	0.11
	Load	1	3.60	3.60	0.05	0.82
	Accel×Load	1	8.05	8.05	0.08	0.78
Early June	Accel	1	279.29	279.29	2.64	0.12
	Load	1	1274.18	1274.18	12.74	<0.01*
	Accel×Load	1	183.68	183.68	1.25	0.28
Late June	Accel	1	0.06	0.06	<0.01	0.98
	Load	1	400.70	400.70	3.88	0.06
	Accel×Load	1	445.50	445.50	7.09	0.01*
July	Accel	1	0.02	0.02	<0.01	0.99
	Load	1	160.78	160.78	1.03	0.32
	Accel×Load	1	2.68	2.68	0.03	0.87
August	Accel	1	141.38	141.38	1.75	0.19
	Load	1	6.72	6.72	0.06	0.80
	Accel×Load	1	12.01	12.01	0.17	0.68
October	Accel	1	156.07	156.07	3.33	0.08
	Load	1	45.15	45.15	0.70	0.41
	Accel×Load	1	10.04	10.04	0.24	0.63
November	Accel	1	77.54	77.54	0.85	0.37
	Load	1	42.98	42.98	0.41	0.53
	Accel×Load	1	18.22	18.22	0.15	0.71
February	Accel	1	12.31	12.31	0.11	0.74
	Load	1	81.60	81.60	0.75	0.39
	Accel×Load	1	6.03	6.03	0.05	0.82

*Significant effects were observed only in the two June experiments.

Table 3. *Summary of byssal thread production assays at Bass Rock, RI, USA*

	Mussel measurements				Environmental conditions	
Start date	Thread production (day^{-1})	GI	CI ($\times 10^{-2}$ g cm^{-3})	*N*	Temperature (°C)	H_s (m)
Oct 23, 2003	4.49±2.42^a	0.09±0.05	0.32±0.08	15	14.13	0.59
Nov 22, 2003	3.25±2.15^a	0.08±0.04	0.32±0.07	19	10.64	0.62
Feb 7, 2004	0.56±0.79^b	0.08±0.03	0.41±0.08	19	2.79	0.81
Feb 17, 2004	0.63±0.67^b	0.07±0.02	0.39±0.10	20	2.33	0.69
Mar 19, 2004	0.93±1.25^b	0.12±0.02	0.58±0.10	20	2.88	0.89
Apr 24, 2004	4.38±3.25^a	0.15±0.03	0.79±0.24	16	7.10	0.65
May 17, 2004	3.66±2.36^a	0.08±0.03	0.48±0.12	20	11.45	0.55
Jun 14, 2004	3.91±2.48^a	0.13±0.02	0.52±0.08	19	14.48	0.58
Jul 12, 2004	3.30±3.17^a	0.10±0.02	0.43±0.08	16	18.78	0.83
ANOVA	$P<0.001$					

Values for mussel data are means ± s.d.

ANOVA value indicates thread production rate differed significantly among assay dates; superscripts denote groupings defined by multiple comparisons testing (Holm–Sidak method, $P<0.05$).

Environmental conditions are means of daily values reported during mussel assay; see text for details.

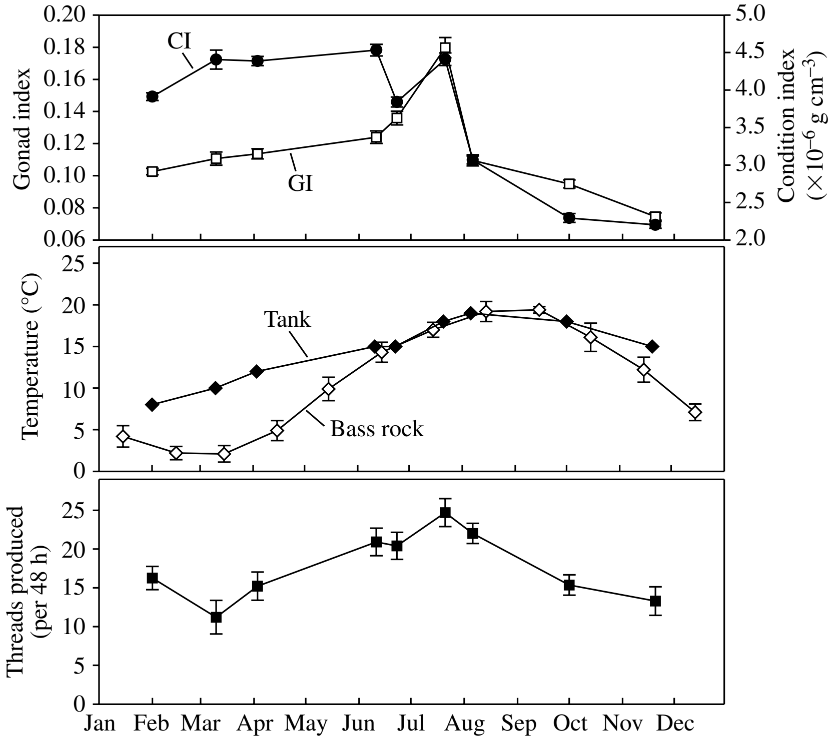

Fig. 6. Summary of mussel condition (gonad index, GI, and condition index, CI), water temperature and thread production for all low flow experiments. Water temperature is shown as both tank temperature and mean (± s.e.m.) bottom temperature at Bass Rock (year 2003–2004). Values for GI, CI and threads produced are means (± s.e.m.) of pooled experimental mussels (N=30–60).

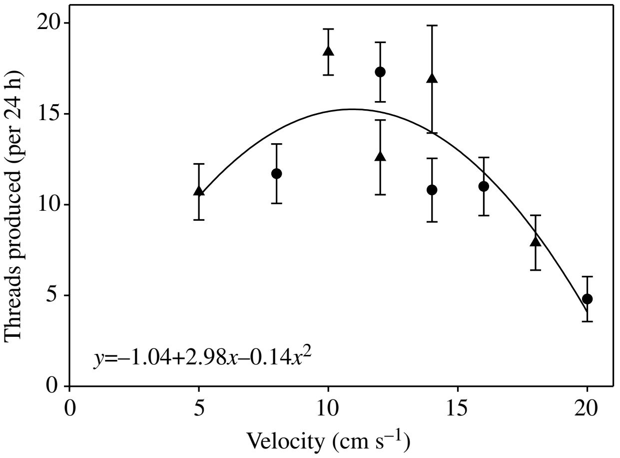

Fig. 7. Mean thread production over 24 h as a function of water velocity in a circulating flume. Values are means ± s.e.m. (N=10 mussels); symbols (circles, triangles) distinguish the two groups of ten mussels used. The equation is a second order polynomial fit to the data (solid line).

proportion of the variation in the dependant variable (59%), with increased temperature correlated with increased thread production. Wave height (H_s) and GI accounted for substantially lower proportions of the variation in thread production (<20%). Thread production was positively correlated with GI, but negatively correlated with wave height. Together, the three variables combine to explain over 90% of the variation in thread production, with maximal values predicted for conditions with elevated temperature, reduced wave action, and high mussel reproductive condition.

Discussion

If wave action were the primary cue influencing attachment strength, one would expect a positive correlation between thread production and at least one of the three aspects of wave action examined in this study, especially during storm seasons. The strongest response was observed for high flow, with a significant decrease in thread production during all seasons. Overall, byssal loading also hindered thread production while acceleration elicited a weak response. In the flume, thread production dropped dramatically at flows greater than 18 cm s^{-1} and thread production in the field assay was also negatively correlated with wave action, confirming laboratory results. Together, these results suggest that increased wave action not only fails to induce thread production, it instead suppresses the mussels' ability to produce threads.

The strong response to increased flow contradicts the results of previous studies on *M. edulis*, most probably because the latter have focused on the differences in thread production at only two velocities (Dolmer and Svane, 1994; Maheo, 1970; Van Winkle, 1970). The flume experiment illustrated that thread production increased concurrently with flow, up to ~10 cm s^{-1}, then decreased as flows continued towards 20 cm s^{-1} thus demonstrating that the relationship between flow and thread production is not linear. Video footage indicates that mussels had difficulty maintaining the proper foot extension to produce threads at higher velocities (>18 cm s^{-1}) due to premature retraction. This inability to maintain contact with the substrate is most probably driving the curvilinear trend. A similar pattern was observed for the zebra mussel, *Dreissena polymorpha*, where thread production peaked at velocities of 20 cm s^{-1} (Clarke and McMahon, 1996a).

The bell curve pattern probably explains discrepancies between this study and other 'positive' flow effects. Depending on which two flow levels are chosen, a positive, neutral, or negative correlation will be observed. This underscores the need to measure at more than two levels to detect relationships described by second order polynomials; more than two points are required to define a peak.

In addition to flow, byssal loading generally decreased

Table 4. *Summary of forward stepwise regression analysis of thread production by Mytilus edulis*

Variable (Step)	Coefficient	Standard coefficient	F	P	Δr^2
Temperature (1)	0.15	0.366	13.56	0.014	0.59
H_s (2)	–6.55	0.549	11.34	0.020	0.18
GI (3)	22.17	–0.500	6.86	0.047	0.13
Constant	3.70				

The dependent variable was mean thread production rate in nine field assays. Condition index (CI) was not selected for entry as an independent variable because of colinearity with GI (gonadal index).

thread production while the effect of acceleration was inconsistent and sporadic across months. These results are not surprising, as previous studies have also found that agitation, the combination of byssal loading and acceleration, produces conflicting responses in mussels. Young (1985) observed a marked increase in thread production following the agitation of *M. edulis*, whereas, in *D. polymorpha*, byssal thread production was found to be both reduced and enhanced in response to agitation (Clarke and McMahon, 1996b; Rajagopal et al., 1996). The inconsistent response to byssal loading across months observed in this study is most probably due to the variable amount of load that was experienced by each individual mussel, which was directly proportional to the number of threads produced by the mussel and placed in tension. Therefore, mussels that produced more threads experienced a higher level of byssal loading than those that produced fewer threads.

While thread production was not enhanced by wave action, it did vary seasonally, corresponding to changes in environmental and physiological conditions. Field experiments indicate that 90% of the variation in thread production can be predicted from changes in temperature, wave height and reproductive condition; temperature explains the largest amount of the variation. Similar observations have been made previously (Allen et al., 1976; Clarke and McMahon, 1996c; Young, 1985) suggesting that temperature may be a better predictor of thread production in mussels than wave action. However, it is unknown whether temperature is the underlying mechanism driving this change or another factor that varies concomitantly with temperature. For example, both nutrient abundance and salinity vary seasonally with sea surface temperature, therefore, either of these factors could be driving the variation in thread production (Carrington, 2002a).

Carrington found that increased tenacity was significantly correlated with increased thread number and proposed that elevated thread production following hurricane season leads to stronger attachment in the winter (Carrington, 2002a). However, this model was based on a regression analysis largely driven by three points; high tenacity was frequently associated with lower thread numbers. Both the laboratory and field data from this study indicate that more threads are being produced when mussels are most weakly attached to the substrate (Fig. 8); these periods coincide with elevated temperature and reproductive effort, and reduced wave height. Therefore, increased tenacity in winter is not the result of increased production as suggested (Carrington, 2002a), but instead may be the product of stronger individual threads that are more decay resistant. Thus, a reduced ‘shelf life’ in summer could reconcile the patterns observed by this study and Carrington (2002a). This suggests that indeed, seasonal variation in byssal thread quality and rates of decay warrant further investigation (Moeser, 2004).

Wave heights have markedly increased over the past 50 years (Bacon and Carter, 1991; Hoozemans and Wiersma, 1992) and, more specifically, the incidence and strength of hurricanes has increased significantly within the past 10 years in the North Atlantic Basin (Goldenberg et al., 2001; Webster et al., 2005). Since hurricane season coincides with the period of weakest attachment, the inability of mussels to respond positively to wave action could severely increase dislodgement. This increase in hurricane activity also occurs concomitantly with an increase in sea surface temperature (Webster et al., 2005), which is represented not only by a change in local temperature, but has also manifested itself as changes in wave height, precipitation and other atmospheric conditions, seasonal patterns, and even nutrient abundance (Helmuth et al., 2005). Because these factors are interconnected, it is difficult to predict how mussels will respond to changes in any single environmental variable (Helmuth et al., 2005).

While our results indicate that thread production is hindered at higher velocities, mussels are still able to securely attach to wave-exposed surfaces during periods of higher flows. When do mussels produce threads? Perhaps, high intertidal flows are

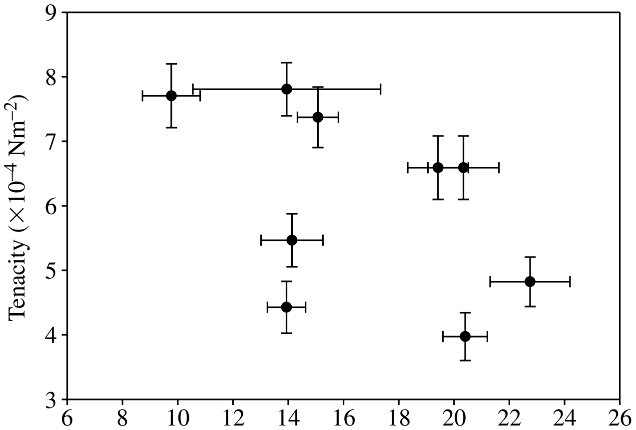

Fig. 8. Mussel tenacity *vs* thread production. Thread production values are the least square means (± s.e.m.) of pooled data from each simulated wave action experiment. Tenacity in the corresponding months was estimated as mean (± s.e.m.) of 6 years of measurements at Bass Rock (see text for details). A linear regression of the data was not significant (P=0.18, r^2=0.24; y=–0.17x+8.89).

significantly dampened within dense mussel beds, or mussels produce threads only when flows are low, such as during slack tides. A more detailed measure of water flow within mussel beds along with observations of thread production in the field would provide insight into this issue.

Although the literature has focused on the integration of wave action and thread production as the primary process affecting mussel attachment strength, this study shows that even modest wave action decreases thread production. More specifically, we have shown that the relationship between flow and thread production is not linear, and that water temperature is the environmental variable that best explains seasonal changes in thread production. Future studies should examine how byssal thread material properties, and their dependence on environmental and physiological conditions, influence mussel attachment strength.

We thank Ben Rutt, Mike Concodello and Trevor Higgins for help with devising and implementing the field thread production experiment. M. Boller, J. Dimond and J. Mello provided helpful advice and logistical support. Funding was provided by NSF in the form of grants OCE-9711893 and OCE-0082605 to E.C.; H.L. was supported by an REU supplement. We are also grateful for the support of the Department of Biological Sciences and the Coastal Fellows Program at the University of Rhode Island. Portions of this study were submitted to the University of Rhode Island by G. Moeser in partial fulfillment of the requirements for the Masters of Science degree.

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