Mechanical properties of the rigid and hydrostatic skeletons of molting blue crabs, *Callinectes sapidus* Rathbun

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Summary

Molting in crustaceans involves significant changes in the structure and function of the exoskeleton as the old cuticle is shed and a new one is secreted. The flimsy new cuticle takes several days to harden and during this time crabs rely on a hydrostatic skeletal support system for support and movement. This change from a rigid to a hydrostatic skeletal support mechanism implies correlated changes in the function, and thus mechanical properties, of the cuticle. In particular, it must change from primarily resisting compression, bending and torsional forces to resisting tension. This study was designed to explore the changes in the mechanical properties of the crustacean cuticle as the animals switch between two distinct skeletal support mechanisms. Samples of cuticle were removed from blue crabs, *Callinectes sapidus*, at 1 h (soft-shell stage), 12 h (paper-shell stage), and 7 days (hard-shell stage) following molting. We measured and compared the flexural stiffness, Young's modulus of elasticity (in tension), and tensile strength for each postmolt stage. We found that the hard-shell cuticle has a flexural stiffness fully four orders of magnitude greater than the soft-shell and paper-shell cuticle. Although the soft-shell cuticle has a Young's modulus significantly lower than that of the paper-shell and hard-shell cuticle, it has the same tensile strength. Thus, the soft-shell and paper-shell cuticles are unable to resist the significant bending forces associated with a rigid skeletal support system, but can resist the tensile forces that characterize hydrostatic support systems. The mechanical properties of the cuticle thus change dramatically during molting in association with the change in function of the cuticle. These results emphasize the significant role that mechanics plays in the evolution of the molting process in arthropods, and possibly other ecdysozoans.

Key words: crab, molting, *Callinectes sapidus,* cuticle, mechanical properties, tensile strength, Young's modulus, flexural stiffness, hydrostatic skeleton.

Introduction

In order to grow, arthropods must undergo the remarkable and risky process of completely shedding their hard external skeleton and secreting a new one. Given that a skeleton is necessary for posture and voluntary movement, it is no surprise that many researchers have assumed that the loss of the rigid exoskeleton temporarily immobilizes molting crustaceans. But many crustaceans and insects are in fact capable of performing normal activities immediately following molting (Olmsted and Baumberger, 1923; Scott and Hepburn, 1976; Lipcius and Herrnkind, 1982; Steger and Caldwell, 1983; Adams and Caldwell, 1990; Cromarty et al., 1991; Katz and Gosline, 1992; Queathem and Full, 1995). In crabs, and probably other arthropods as well, mobility is maintained during molting by switching to a hydrostatic skeletal support system (Taylor and Kier, 2003; Taylor and Kier, 2006). Rigid and hydrostatic skeletons differ greatly in their structure and mechanisms of support. Thus, alternation between the two skeletal types requires significant structural and functional changes in the cuticle.

Rigid skeletal support systems are common in vertebrates, arthropods, echinoderms and other phyla. Antagonistic muscles insert on stiff skeletal elements that move relative to one another at joints and can function as levers that may amplify either the force or the displacement of muscle contraction. The forces of muscle contraction are thus transmitted through the stiff elements as compressional, torsional and bending stresses (Wainwright, 1982) that must be resisted by the rigid elements in order to avoid failure. In crabs, the body is typically held above the substratum by multiple jointed legs. The merus (the fourth, and often longest, segment) of the walking leg is extended laterally and horizontal with the body, while the last leg segments extend vertically to the substrate (Hahn and LaBarbara, 1993). This body posture places bending and torsional stresses on the merus, and compressional stresses on the last limb segments, so that limb failure typically occurs by local buckling (Currey, 1967; Hahn and LaBarbara, 1993). Rigid skeletons thus function primarily by resisting compressional, bending and torsional stresses.

Hydrostatic skeletons are common in soft-bodied invertebrates including polyps, such as sea anemones, and many types of worm. Classical hydrostatic skeletons have no rigid elements, are typically cylindrical in shape, and consist of a flexible, muscular body wall surrounding a liquid-filled cavity (Chapman, 1958; Trueman, 1975; Gutmann, 1981; Wainwright, 1970; Wainwright, 1982; Wainwright et al., 1976). The body wall typically includes antagonistic longitudinal and circumferential muscle layers and is reinforced with connective tissue fibers. The forces of muscle contraction are transmitted through the essentially incompressible fluid, resulting in an increase in the internal hydrostatic pressure and tension in the body wall. To prevent changes in body shape, the body wall must resist this tension (Wainwright, 1970). The structure and mechanical properties of the body wall therefore control shape changes of the animal by resisting tensile forces (Harris and Crofton, 1957; Clark and Cowey, 1958).

The crustacean skeleton alternates between these two dramatically different forms of skeletal support each time molting occurs. During molting, the animal first secretes a new cuticle beneath the old exoskeleton. Then ecdysis begins, during which the animal draws in water through the mouth, breaks the carapace, withdraws from the old exoskeleton (exuviation), and continues to absorb water until the new, soft cuticle is inflated to a larger size. It then takes several days before the new cuticle hardens (see Herrick, 1895; Drach, 1939; Richards, 1951; Passano, 1960; Aiken, 1980; Skinner, 1985). Thus, immediately after exuviation, crabs are soft, inflated with water, and the cuticle is in tension, resembling other animals with hydrostatic skeletons. The muscular arrangement, however, remains unchanged.

Concurrent with this dramatic change in the mechanical function of the cuticle during the molt cycle are the remarkable changes that occur in its structure (Roer and Dillaman, 1993). The cuticle changes from a rigid and tough material that requires significant force to bend and break, to a flimsy membrane that deforms as easily as plastic wrap. The cuticle is composed of four layers: epicuticle, exocuticle, endocuticle and membranous layer, in order from outermost to innermost. These layers are composed of a chitin–protein matrix and calcium carbonate (Roer and Dillaman, 1984). Before ecdysis occurs, the new epicuticle and exocuticle layers are secreted by the underlying hypodermis. These layers begin hardening, by cross-linking, once exuviation is complete (Drach, 1939; Dennell, 1947; Travis, 1963). Thus, for several hours following exuviation, the cuticle is highly flexible and soft. This period is referred to as the soft-shell stage in blue crabs. Within just a few hours of ecdysis, the innermost and thickest layer of the cuticle, the endocuticle, is secreted and begins calcification (Travis, 1957; Travis, 1963; Travis, 1965). At approximately 12 h after ecdysis, tanning and mineralization begins and the cuticle attains the texture of paper. In blue crabs, this is referred to as the paper-shell stage. The calcification process continues until the entire cuticle is secreted. The deposition of calcium carbonate, protein and chitin continues for up to 30 days postmolt (Dendinger and Alterman, 1983). Crabs are then referred to as being in the hard-shell stage until the next molt. This elaborate process of regeneration and hardening of the cuticle during each molt provides the structural changes that both require and facilitate the switch between rigid and hydrostatic support mechanisms.

The changes in structure and function of the cuticle imply correlated changes in the mechanical properties of the cuticle. As the cuticle transitions from rigid to soft and flexible during molting, it must also change from primarily resisting compressive, torsional and bending forces to primarily resisting tensile forces. Though the mechanical properties of some crustacean cuticles have been measured previously (Hepburn et al., 1975; Joffe et al., 1975a; Joffe et al., 1975b; Hepburn and Chandler, 1976; Currey et al., 1982; Dendinger and Alterman, 1983; Palmer et al., 1999; Dutil et al., 2000), few studies have measured the mechanical properties throughout the molt cycle (Dendinger and Alterman, 1983; Dutil et al., 2000). Both of these studies found significant changes in tensile strength and elastic modulus of the cuticle immediately following molting. In this study, we document the changes in the mechanical properties of the cuticle associated with its change from a rigid to a hydrostatic skeleton. We measured the flexural stiffness, Young's modulus of elasticity (in tension) and tensile strength of the cuticle of the blue crab, *Callinectes sapidus*, at a series of postmolt cuticle stages. We discuss these mechanical properties in the context of the role of the cuticle in skeletal support and movement at each stage.

Materials and methods
Animals

The blue crab, *Callinectes sapidus* Rathbun, was analyzed in this study because of its large size and because external changes in coloration provide an indication of the timing of the next molt (Otwell, 1980). Male and female 'peeler' crabs (within 2–3 days of molt) ranging from 70 to 85 mm premolt carapace width were obtained from O'Neals Sea Harvest (Wanchese, NC, USA). Crabs were transported to the University of North Carolina at Chapel Hill where they were maintained in individual artificial seawater aquaria (280 mm × 172 mm × 203 mm) at a temperature of 19°C and a salinity of 15–20 p.p.t. (Instant Ocean Artifical Seawater, Aquarium Systems Inc., Mentor, OH, USA). Water changes were performed consistently for all crabs every 2 days. Animals were checked every 2 h for the onset of exuviation. The time postmolt was calculated from the time exuviation was complete. Cuticle samples were taken from individual crabs at three different times postmolt: 1 h (soft-shell stage), 12 h (paper-shell stage) and 7 days (hard-shell stage). The animals were not fed before or during experiments.

Cuticle samples

Both chelipeds were autotomized from crabs at the three specified postmolt stages. Limb autotomy is a natural, non-lethal defense mechanism that can be quickly and easily induced by either applying pressure or making a small puncture with a needle near the merus-basis joint. One cheliped was used for tensile testing and the other was used for bending tests. If the chelipeds differed in size, the larger one was used for tension tests (the larger size facilitated the preparation of the samples for tensile testing) and the smaller one for bending tests; otherwise, they were selected at random. Rectangular samples of cuticle were cut, using a razor blade or scissors, from the flat, dorsal surface of the merus segment. For bending tests, samples

were cut longitudinally, while samples for tensile tests were cut in the hoop direction (i.e. circumferentially). This allowed the largest piece of material to be cut and the loading during the tests to be performed in an orientation that was appropriate for loading in the animal. It is possible that the cuticle is anisotropic and thus our results may differ from mechanical properties measured in other directions. Cuticle samples removed for bending tests were approximately 5–9 mm wide and 15–25 mm long and those for tensile tests were approximately 5 mm wide and 10 mm long. The hypodermis was carefully removed from all samples with the edge of a razor blade under a microscope. For bending tests, cuticle samples were tested immediately. Because we had limited access to the tensile testing instrumentation, cuticle samples for tensile tests were immediately placed in vials of seawater and stored in a refrigerator until testing could be performed. Samples were stored in this manner to avoid the effects of chemical fixatives on the mechanical properties. Samples were kept for no longer than 3 weeks before testing. Prior to and during all tests, specimens were kept moistened with seawater since hydration state affects the mechanical properties (Hepburn et al., 1975; Joffe et al., 1975b).

Bending tests

Soft and paper cuticle apparatus

A 3-point bending apparatus was constructed that employed two no. 7 size stainless steel insect pins (0.70 mm diameter) as supports while force was applied to the center of the sample with a force transducer (Fig. 1). The insect pins were attached to the side of a solid brass bar (100 mm × 19 mm × 19 mm) so that they extended vertically, 10.19 mm apart. Cuticle samples were placed against the insect pins with the epicuticle surface facing away from the force transducer. This ensemble was then placed in a square plastic box (11 cm × 11 cm × 3 cm) filled with seawater on the stage of a dissecting microscope. Two force transducers that incorporated silicon strain gauge elements were used (AE-801 Sensor Element, SensorOne Technologies Corp., Sausalito, CA, USA). A no. 0 stainless steel insect pin (0.35 mm diameter) was epoxied to the silicon beam of the sensor element and bent at a 90° angle so that the head pressed against the cuticle sample. The sensitivity of the force transducer used for the soft cuticle samples was increased by lengthening the moment arm with a 90 mm long, 0.35 mm diameter stainless steel wire attached to the insect pin. The force transducer was mounted on a 3-axis micromanipulator placed adjacent to the microscope. The micromanipulator was used to advance incrementally the pin attached to the force transducer in the center of the sample. The displacement of the cuticle sample and any displacement of the force transducer were measured with an ocular micrometer. At each displacement, the force and distance of displacement were recorded. The total displacement was less than 10% of the sample length.

The force transducers were connected to a custom-built bridge/amplifier and the signals were fed to an analog-to-digital conversion unit (DI-700, Dataq Instruments, Akron, OH, USA) connected to a computer. Recordings of force were collected at 488 Hz using data acquisition software (WinDaq/Lite, Dataq Instruments). The data were analyzed using Dataq Software.
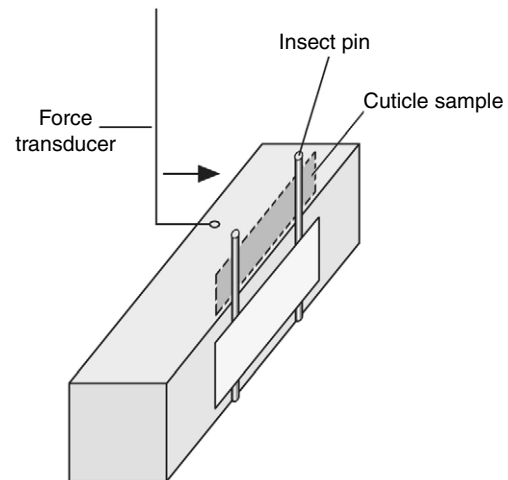

Fig. 1. Diagram of the 3-point bending apparatus used for soft- and paper-shell cuticle samples. The arrow indicates the direction of movement of the loading probe, which was attached to the force transducer and contacted the sample at the midpoint between the two insect pins.

The force transducers were calibrated before and after each series of experiments with objects of known weight. The ability of the 3-point bending apparatus to predict the Young's modulus of samples was confirmed using plastic shim stock (Artus Corp., Englewood, CA, USA) of known modulus and a range of thicknesses: 0.01, 0.02, 0.025, 0.04 and 0.05 mm.

Hard cuticle apparatus

A tensometer (Hounsfield Tensometer, Tensometer Limited, Croydon, UK) was adapted for 3-point bending tests (Fig. 2). The moveable grip was modified by adding two horizontal bars, 19.8 mm apart. A third horizontal bar was attached to the beam of a force transducer (Fort 250, Precision Instruments, Sarasota, FL, USA) clamped to the stationary grip. The cuticle sample was inserted vertically between the two horizontal bars with the epicuticle facing away from the transducer. The three horizontal bars were machined so that the sample rested against a sharp 90° ridge on each. The moveable grip was advanced incrementally and the distance between the two grips (displacement) was measured using an ocular micrometer attached to a free-standing surgical microscope. The force transducer was connected to the same equipment and analyzed using the same software as described for the soft and paper cuticle above. Additionally, calibrations of the force transducer and verification of the 3-point bending apparatus were made in the same manner as for the apparatus used for the soft and paper cuticle but with a range of heavier calibration weights and with plastic shim stock of the following thicknesses: 0.25, 0.31, 0.4, 0.5, 0.6 and 0.75 mm.

Tension tests

Tension tests were conducted on a vertical, table-top universal materials testing instrument (Lloyd Instruments LR5K Plus, Ametek, Inc., Fareham, UK). Each end of a cuticle sample was sandwiched between two 19 mm × 19 mm square aluminium grips using cyanoacrylate adhesive to augment the
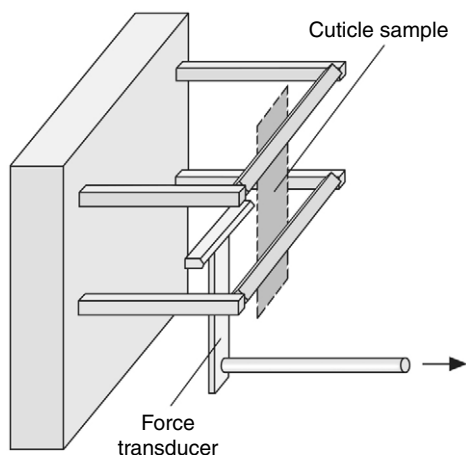
Fig. 2. Diagram of the 3-point bending apparatus used for hard-shell cuticle samples. The arrow indicates the direction of movement of the force transducer with the attached bar used to load the sample at the midpoint between the two fixed bars.

attachment. Soft and paper cuticle samples were placed in 0.90 mm thick aluminium grips. Hard cuticle samples required sturdier grips, 2.25 mm thick, with a roughened attachment surface. Samples were placed in the material testing apparatus so that they were stretched in the hoop direction. Tests were run using a 500 N load cell at a rate of 5 mm min^{-1} and force and displacement data were acquired using the stock Lloyd software.

Analysis
Bending

Flexural stiffness, *EI*, was calculated using the equation for 3-point bending:

$$y = \frac{Fl}{48EI}, \tag{1}$$

where *y* is the deflection of the sample, *F* is the applied force, *l* is the distance between pins or beams, and *EI* is the composite variable for flexural stiffness (Young's modulus, *E*, multiplied by the second moment of area, *I*). *EI* was calculated for each displacement for individual cuticle samples and then averaged. The values of *EI* for each cuticle sample were then averaged for each postmolt stage (soft, paper and hard) and compared across stages using non-parametric analyses (Zar, 1999).

Tension

The force and displacement data obtained for each tension test were converted to stress and strain for analysis. Stress, σ, was calculated as engineering stress:

$$\sigma = \frac{F}{A}, \tag{2}$$

where *F* is the instantaneous force applied and *A* is the initial cross-sectional area of the sample perpendicular to the applied force. To calculate the cross-sectional area of hard cuticle samples, the width and thickness were measured using a digital

caliper. For each cuticle sample, 5–10 measurements were made and averaged. The thickness of the hard cuticle averaged 0.28 mm (s.d.=0.14 mm, *N*=21). Because the soft and paper cuticle samples were too thin to measure accurately with calipers, cuticle thickness was measured using laser scanning confocal microscopy. The cuticle samples were immersed in poly-L-lyseine for 10–30 min to aid the adhesion of fluorescent yellow green latex beads (L2153, Sigma-Aldrich, Inc., St Louis, MO, USA) to the inner and outer cuticle surface. Following immersion in poly-L-lyseine, the samples were dipped in a suspension of the latex beads, placed on microscope slides and coverslipped. Thickness was measured from a *Z*-axis series in the confocal microscope as the distance between the beads on the inner and outer surfaces of the cuticle sample. For each cuticle sample, 10–30 measurements were taken and then averaged. The mean thickness of the soft cuticle was 20 μm (s.d.=2.9 μm, *N*=9) and that of the paper cuticle was 30 μm (s.d.=13 μm, *N*=15).

Strain, ε, was calculated as engineering strain:

$$\epsilon = \frac{\Delta L}{L_0}, \tag{3}$$

where ΔL is the change in length of the sample and L_0 is the initial sample length. The use of engineering strain rather than true strain should not cause significant error since the measured strains are all relatively low (less than 12%).

A stress–strain plot was created for each cuticle sample, from which the Young's modulus of elasticity (stiffness), the tensile breaking strength and the work to failure were obtained. The Young's modulus of the material in tension is the ratio of stress to strain (i.e. the slope of the plot). For non-Hookean materials (those that do not show a linear relationship between stress and strain), the stiffness can be estimated with a tangent modulus, measured as the slope of the linear portion of the stress–strain plot between 10 and 50% of the strain at failure (Hepburn and Joffe, 1974). The tensile strength of a material is the stress at failure. The work to failure was estimated by calculating the area under the stress–strain curve up to the point of failure. The material properties were averaged among samples of each postmolt stage (soft, paper and hard) and compared across stages using JMP IN 5.1 statistical software. Data for the modulus and strength were determined to have a normal distribution by the Shapiro–Wilcoxon test, so an ANCOVA was used to test for differences among the cuticle samples with storage time as a covariate. The estimated work to failure was compared among stages using non-parametric statistics (Zar, 1999).

Results
Bending

Soft, paper and hard cuticle samples had a mean flexural stiffness of 2.0×10^{-9} N m^2 (s.d.=2.3×10^{-9} N m^2, *N*=10), 7.2×10^{-9} N m^2 (s.d.=8.6×10^{-9} N m^2, *N*=10) and 1.8×10^{-5} N m^2 (s.d.=2.0×10^{-5} N m^2, *N*=12), respectively (Fig. 3). Thus, the hard cuticle has a flexural stiffness that is four orders of magnitude greater than that of both the paper and soft cuticle (Kruskal–Wallis, χ^2=13.8, *P*<0.001; *Q*=2.39, *P*<0.05; *N*=12, 10 and 10, respectively). The measured flexural stiffness of soft and paper cuticle samples were not statistically different.
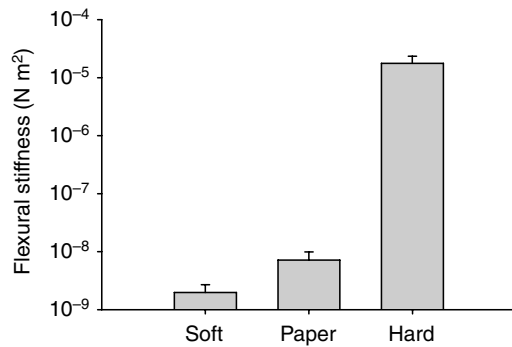
Fig. 3. Mean flexural stiffness, EI, of soft, paper and hard cuticle (N=10, 10 and 12, respectively). Note that EI is plotted on a logarithmic scale: EI of the hard cuticle samples is four orders of magnitude higher than that of the soft or paper cuticle samples. Error bars show s.e.m.

Fig. 5. Mean Young's moduli, E, of soft, paper and hard cuticle (N=15, 18 and 12, respectively). The Young's moduli of the hard and paper cuticle samples were similar and significantly greater than that of the soft cuticle samples. Error bars show s.e.m.
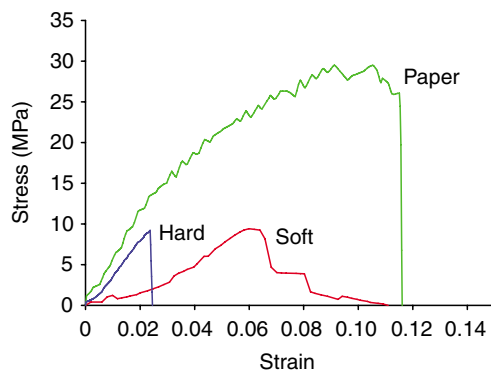

Fig. 4. A typical stress–strain plot for the soft, paper and hard cuticle samples. Note the similarity in modulus (slope) of the paper and hard cuticle samples, which were significantly stiffer than the soft cuticle samples. The tensile strength (stress at failure) of the soft, paper and hard cuticles were not significantly different.

Fig. 6. Mean tensile strength of soft, paper and hard cuticle (N=15, 19 and 12, respectively). The tensile strengths of the three cuticle stages were similar. Error bars show s.e.m.

Tension

The stress–strain plots for soft, paper and hard cuticle all show an approximately linear portion and a distinct point of failure (Fig. 4). The mean Young's modulus of the soft cuticle samples was 137 MPa (s.d.=91 MPa, N=15), which was significantly lower than that of the paper-stage cuticle (274 MPa, s.d.=186 MPa, N=18) and the hard cuticle (318 MPa, s.d.=133 MPa, N=12; ANCOVA, P<0.05; Tukey, P<0.05; N=12; Fig. 5). The Young's moduli of paper and hard cuticle are not significantly different. There was no effect of storage time on the moduli (ANCOVA, P>0.05). Soft cuticle samples tended to fail by tearing while both paper and hard cuticle samples failed by fracture. The mean tensile strengths of the soft, paper and hard cuticle were 9.8 MPa (s.d.=3.7 MPa, N=15), 16 MPa (s.d.=11 MPa, N=19) and 9.9 MPa (s.d.=6.6 MPa, N=12), respectively (Fig. 6). The tensile strength of the paper cuticle appears to be greater than that of the soft and hard cuticle, and there is a significant difference among the samples (ANCOVA, P<0.05), but the effect was too weak to be picked up by a Tukey test (P>0.05, N=12). Furthermore, there was a slight effect of storage time on the strength of the cuticle samples (ANCOVA, P<0.05). Finally, the work to failure of the
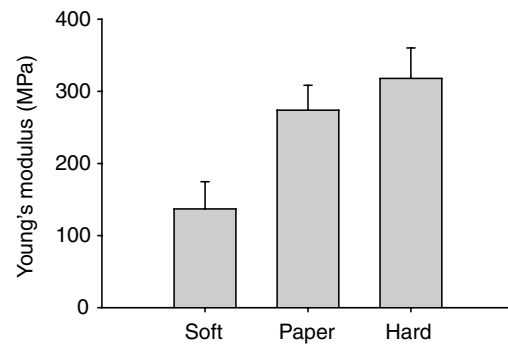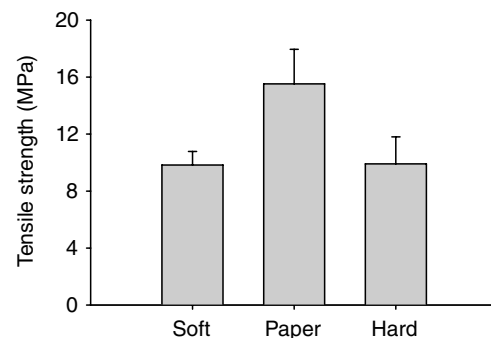
hard cuticle (0.17 MPa, s.d.=0.13 MPa, N=12) was significantly lower than that for both the soft (0.71 MPa, s.d.=0.76 MPa, N=15) and paper (0.81 MPa, s.d.=0.85 MPa, N=18) cuticle (Kruskal–Wallis, χ^2=12.7, P<0.01; Q=2.39, P<0.05; N=15, 18 and 12, respectively).

Discussion

During molting, crustaceans rely on a hydrostatic skeletal support system, which differs greatly from their characteristic rigid exoskeleton of intermolt periods; hydrostatic skeletons use a fluid to transmit forces and the external container (typically the body wall) is loaded primarily in tension. In order for crabs to alternately function with each type of skeleton, the cuticle must be able to withstand compressional, bending and torsional stresses during intermolt periods and tensile stresses during molting. Although the cuticle during the soft-shell stage is too flexible to support the compressional and bending forces exerted during locomotion, our results demonstrate that it can bear the same magnitude of tensile stresses as the much stiffer intermolt cuticle.

For the first 12 h following ecdysis, the soft cuticle has a remarkably low flexural stiffness (2×10^{-9} to 7×10^{-9} N m^2). When handled, soft cuticle folds easily under its own weight and obviously cannot resist significant bending forces. This presents a fundamental problem for functioning as a typical rigid exoskeleton because movement and muscular antagonism require

that the cuticle resists the bending forces associated with muscle contraction. The claw muscles of decapod crustaceans are capable of producing large stresses, as high as 400–2000 kN m^{-2} during intermolt (Josephson, 1993). During molting, intrinsic or extrinsic factors may reduce the force production of some muscles in order to prevent damage to muscle fibers or cuticle. The claw muscles undergo significant atrophy prior to molting (Skinner, 1966; Mykles and Skinner, 1981; Mykles and Skinner, 1982; Mykles and Skinner, 1990) but they are still capable of producing force following ecdysis, and thus remain functional (West et al., 1995; West, 1997). This is important since the exuviation process itself requires repeated, forceful movements in order to pull the appendages out of the old exoskeleton (Travis, 1954; Lipcius and Herrnkind, 1982; Phlippen et al., 2000). Deformation in the cuticle can be seen during this active process (J.R.A.T., unpublished observation) and the increased internal hydrostatic pressure resulting from postmolt inflation (deFur et al., 1985; Taylor and Kier, 2003) is necessary to provide resistance to muscle contraction. Indeed, hydrostatic skeletal support is used by the blue crab during the first 12 h following ecdysis (Taylor and Kier, 2003).

As the cuticle hardens during the first week after ecdysis, the flexural stiffness increases by four orders of magnitude (1.8×10^{-5} N m^2). This large increase in flexural stiffness correlates with the progression of the mineralization process (Vigh and Dendinger, 1982; Dendinger and Alterman, 1983). By 7 days following ecdysis the hard cuticle is capable of resisting significant bending, torsional and compressional forces. At this time, the cuticle, rather than the fluid, transmits the forces of muscle contraction and the animal once again functions using a rigid skeletal support system (Taylor and Kier, 2003).

The tensile properties of the cuticle also change significantly as crabs switch between rigid and hydrostatic skeletons. The tensile stiffness, or Young's modulus, of the soft cuticle within an hour of exuviation is only 132 MPa, but increases significantly to 379 MPa 12 h later during the paper stage and stabilizes at 361 MPa a week later during the hard stage. We are not aware of measurements of tensile stiffness for newly molted animals, but the tensile stiffness we observed for the hard cuticle of the chelipeds is similar to that reported for the carapace of the crab *Scylla serrata* (481 MPa) (Hepburn et al., 1975) and the carapace of the prawn *Panaeus mondon* (461–549 MPa) (Joffe et al., 1975b). A similar pattern of a rapid increase in flexural stiffness during the first 12 h following ecdysis has also been observed in locust cuticle (Hepburn and Joffe, 1974).

This difference in tensile stiffness between the soft cuticle and the paper and hard cuticles reflects the function of the cuticle during the molting process. During ecdysis, the animal inflates with water and the soft new cuticle is stretched to accommodate the requisite size increase that occurs at each molt. Once ecdysis is complete further stretching may be resisted as the stiffness of the cuticle increases. The increase in Young's modulus observed in the paper stage cuticle is probably associated with calcification and cross-linking of cuticle proteins (Dillaman et al., 2005). Calcium carbonate deposition continues throughout the paper stage, adding stiffness to the cuticle, but levels off after 48 h (Vigh and Dendinger, 1982).

It is striking that the tensile strengths of the soft and paper cuticle are the same as that of the much more robust hard cuticle, ranging from 10 to 15 MPa. The significantly greater cross-linking and mineralization of the hard cuticle do not afford it any greater tensile strength than the soft cuticle. Indeed, the soft and paper cuticles absorb significantly more energy before breaking than the hard cuticle, with work to failure values of 0.71, 0.81 and 0.17 MPa, respectively. These values of tensile strength are slightly less than those found in the hard cuticle of the merus of the crab *Scylla serrata* (30 MPa) (Hepburn et al., 1975) and the carapace of the prawn *Penaeus mondon* (18–28 MPa) (Joffe et al., 1975b). They are similar to previous measurements of the carapace of blue crabs (5.6–15 MPa), despite the fact that they were taken from the carapace rather than the chelipeds and were frozen before testing (Dendinger and Alterman, 1983).

In general, the soft- and paper-shell stage cuticles are incapable of resisting compressional and bending forces but function well in resisting tensile forces. For comparison, the tensile strengths of soft and paper cuticles are greater than concrete brick (5.0 MPa) and are the same order of magnitude as abductin (10 MPa) (Vogel, 2003). The tensile stiffness of soft cuticle approximates that of mussel byssal thread (100 MPa) (Vogel, 2003).

The typical stresses in the cheliped due to the hydrostatic pressure experienced by soft-shell and paper-shell animals in this study were estimated to be 0.86 MPa and 0.16 MPa, respectively. These estimates indicate that soft-shell crabs operate with a safety factor of approximately 10, while paper-shell crabs operate with a larger safety factor of approximately 100. Thus, the risk of tensile failure of the soft cuticle during movement in hydrostatically supported crabs is low.

The changes in the mechanical properties of the cuticle, as it transitions from rigid to soft during the molt cycle, may affect the locomotor ability of animals. For instance, skeletal stiffness is known to affect the jumping ability of the African desert locust (Scott and Hepburn, 1976; Katz and Gosline, 1992), which can vary as much as twofold during the molt cycle (Queathem and Full, 1995). Likewise, changes in the cuticle associated with the shift to hydrostatic skeletal support are likely to affect locomotion in crabs. This could potentially have significant effects on the ability of an animal to escape predators and find shelter during this critical period (Woodbury, 1986).

Molt-induced changes in the structure and mechanical properties of the cuticle may vary among arthropods, in part due to differences in the cuticle that correlate with habitat. For example, as crabs evolved adaptations to life on land, the cuticle became thicker and more heavily calcified. In addition, crustacean cuticle differs from insect cuticle, which is tanned but not calcified, although the material properties of some insects and crustaceans are reported to be similar (Joffe et al., 1975b). A more extensive study of the mechanical properties in other groups of arthropods and other molting phyla may provide new insights into the mechanics of the molting process and reveal additional important characteristics relevant to the evolution of ecdysozoans.

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