Hydrostatic Skeletons in the Crustacea: Support During Molting in an Aquatic and a Terrestrial Crab

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology.

Chapel Hill
2007

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ABSTRACT

JENNIFER REBECCA AMY TAYLOR: Hydrostatic Skeletons in the Crustacea: Support During Molting in an Aquatic and a Terrestrial Crab
(Under the direction of William M. Kier)

All animals require a skeletal support system for posture and locomotion. Arthropods, however, repeatedly shed their rigid exoskeleton in order to grow, yet they maintain shape and mobility during these periods. My research focuses on this apparent paradox and suggests that crabs, and possibly all arthropods, alternate between a rigid and a hydrostatic (fluid-based) skeleton in order to remain functional during molting.

I tested for the use of hydrostatic skeletal support in blue crabs, *Callinectes sapidus*, by simultaneously measuring internal hydrostatic pressure and force of claw adduction. I found a strong correlation between force and hydrostatic pressure in soft-shell crabs, but not in hard-shell crabs, which is consistent with the use of hydrostatic support during molting.

Switching skeletons requires a change in function of the cuticle, from resisting primarily bending, compression, and torsion, to resisting tension. This change in function implies correlated changes in the properties of the cuticle. I tested the mechanical properties of the cuticle throughout the molt cycle of *C. sapidus* and found that the flexural and tensile stiffness is greater in hard cuticle than soft cuticle, but the tensile strength is the same.

The blackback land crab, *Gecarcinus lateralis*, does not molt in water and inflates its gut with air during molting, which may serve as a support mechanism. I simultaneously measured the force of claw flexion, hydrostatic pressure within the claw, and gas pressure within the gut. I

obtained a strong correlation between all three measurements, which suggests that the gas helps maintain turgidity throughout the body, and thus acts as a critical component of the skeleton.

Rigid and hydrostatic skeletons operate according to different principles and each is likely to be influenced by scale in distinct ways. Using morphological techniques, I found that cuticle thickness scales isometrically for rigid skeletons but allometrically for hydrostatic skeletons, suggesting that both play a role in determining growth to maximum size in crabs.

This research provides novel insights into how skeletal support systems influence the way in which animals are built, develop, and function.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. William Kier, for making my graduate studies so rewarding. His amazing personality, expertise, and unfaltering dedication to students make him an exceptional mentor. I could not have achieved my goals without his guidance, patience, encouragement, and moral and financial support. It has been the greatest pleasure having him as a mentor and I am eternally grateful for all that he has done for me.

I thank my current and former committee members: Dr. Kenneth Lohmann, Dr. Joel Kingsolver, Dr. David Pfennig, Dr. Thomas Wolcott, and Dr. Robert Podolsky. They all provided insightful contributions to my research and I am grateful for the time and effort they dedicated to my development as a researcher.

This research was made possible by O'Neals Sea Harvest, who generously provided me with peeler crabs every summer. I also thank Hector Horta and his son Hector Horta Cruz for help with collecting land crabs from Puerto Rico. Several undergraduates, particularly Anna Cruz and Cassidy Henegar, have helped keep the crabs happy over the years.

I thank Jack Hebrank for sharing creative ideas and graciously dedicating several weekends so that I could use his mechanical testing rig. I thank Dr. Tony Perdue for training me to use confocal laser microscopy, Katya Prince for writing the LabView program, and Dr. Louise Roth and Dr. Karin Pfennig for statistical advice. I also thank Dr. Sandra Hoeflich for her kindness and support over the years, and for contributing greatly to my development in areas other than science.

My time at UNC Chapel Hill has been rewarding because of the many great graduate students I have had the pleasure of knowing. I thank them all for making graduate school fun and interesting. I would especially like to thank the other Kier Lab members, Dr. Joe Thompson, Dr. Diara Spain, Ted Uyeno, and Sonia Guarda, for sharing creative ideas, equipment, and friendship throughout the years. I thank Dr. Jon Allen, Dr. Mike Baltzley, and Dr. George Harper for friendship and for dedicating time to editing manuscripts and grant applications. I offer special thanks to Amber Rice, Eileen McDaniel, Lisa Davenport, and Damon Jacobs for their friendship.

This research was supported by grants from the Padi Foundation, Sigma Xi, and The Graduate School at UNC Chapel Hill. Additional sources of funding were NSF and DARPA grants to William Kier.

This dissertation includes material from Taylor and Kier, 2003 and 2006. The first paper is used courtesy of the American Association for the Advancement of Science.

I am especially grateful to my husband, Vladimir Rynkov, for always being there for me. In addition to the unconditional love, support, and encouragement he has given me over the years, he has also provided "crabsitting" services for me many times. Finally, I would like to thank my mom Kay, and my siblings Sherri, Tom, Tammy, Laura, and Mike.

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LIST OF ABBREVIATIONS AND SYMBOLS

A/D	analog to digital
ANCOVA	analysis of covariance
ANOVA	analysis of variance
n	number of samples
C	celsius
cm	centimeter
g	grams
Hz	hertz
h	hour
kNm^{-2}	kilonewton per square meter
MPa	megapascal
min	minute
mm	millimeter
N	newton
Nm^2	newton square meter
Pa	pascal
ppt	parts per thousand
S.D.	standard deviation
SEM	standard error of the mean
sec	second
μm	micrometer
R5	right pereopod 5

L5	left pereopod 5
CA	carapace
LC	left cheliped
L2	left pereopod 2
EI	flexural stiffness
E	Young's modulus of elasticity
I	second moment of area
σ	engineering stress
ε	engineering strain

CHAPTER I

INTRODUCTION

General

In order to grow, arthropods must embark on the remarkable and risky process of molting. Molting involves secreting a new cuticle beneath the exoskeleton, completely shedding the old exoskeleton, inflating to a larger size, and then subsequently hardening the newly formed skeleton (see below). Animals may remain soft for several days until the new skeleton hardens. Thus, arthropods essentially switch from being supported by a rigid armor that requires significant force to break, to being supported by a flaccid skeleton that deforms as easily as plastic wrap. It has often been assumed that crustaceans are inactive and immobile during this time (Passano, 1960; Bliss, 1962; Ryer et al., 1997). In fact, using only the thin, flexible new shell, newly molted animals are able to perform a variety of activities. For instance, newly molted lobsters readily perform an escape response involving forceful tail flips (Cromarty et al., 1991) and newly molted stomatopods often display meral spread threats or flee when their cavities are approached by conspecifics (Steger and Caldwell, 1983; Adams and Caldwell, 1990). Some soft-shell grapsid crabs move about just as actively and rapidly as hard-shell crabs (Olmsted and Baumberger, 1923). Thus, it is clear that crustaceans maintain significant locomotor abilities despite the loss of their rigid exoskeleton.

The ability of crustaceans to maintain forceful movement and locomotion without a rigid skeleton presents a functional paradox. A skeleton is necessary for the creation of movement since it is the structure to which muscles attach and pull on (Schmidt-Nielsen, 1997). The skeleton transmits force, provides for muscular antagonism, resists compression, and amplifies the force, velocity, and displacement of muscle contraction. Without these functions, muscle shortening would result in deformation but no movement. Crustaceans nevertheless maintain the ability to move immediately following molting.

This dissertation describes how crabs support themselves during molting by switching to a hydrostatic (fluid-based) skeleton. The body of crustaceans during the postmolt stage is reminiscent of an animal with a hydrostatic skeleton: they have a soft, flexible cuticle that surrounds a body inflated with a constant volume of fluid, and antagonistic muscle groups. Thus, they possess the characteristics of a hydrostatic skeleton. The possibility of hydrostatic support in crustaceans was mentioned briefly by Drach (1939), Lochhead (1961), and deFur et al. (1985), but has never been examined directly. Some measurements of internal hydrostatic pressure changes have been made throughout the molt cycle in the context of osmotic regulation and a significant increase in pressure was observed during ecdysis (deFur et al., 1985). But no test of the hypothesis that postmolt crustaceans depend on a hydrostatic skeleton for support and movement has been performed.

In order to understand how this shift in skeletal support may occur in crabs, it is necessary to review the molting process and principles of hydrostatic skeletal support in greater detail.

Molting

For all animals with an external cuticle, growth can only occur if the cuticle is shed and a new one is secreted. Molting is thought to have evolved only once during the evolution of animals, and, therefore, all molting animals have been grouped into the clade Ecdysozoa, which includes arthropods, tardigrades, onycophorans, priapulids, kinorhynchs, nematodes, and nematomorphs (Aguinaldo et al., 1997; Ewer, 2005; Pechenik, 2005). The process of molting has been the focus of much research, especially on arthropods (see Herrick, 1895; Drach, 1939; Richards, 1951; Passano, 1960; Aiken, 1980; Skinner, 1985).

The structure of the crustacean cuticle is conserved among species and is similar to that of insects. It is composed of four distinct layers; from outermost to innermost, these are: epicuticle, exocuticle, endocuticle, and membranous layer. The epi- and exo-cuticle layers are calcified following secretion, while the thicker endocuticle is calcified as it is secreted (see Richards, 1951; Neville, 1975; Skinner, 1985; Horst and Freeman, 1993). The membranous layer is secreted last and is not calcified.

Molting is a complex process that is controlled hormonally (see Skinner, 1985). In brief, a molting hormone, ecdysone, is secreted, but repressed by a molt inhibiting hormone (MIH). For reasons that are not well-understood, MIH secretion becomes limited, resulting in increased ecdysone concentration that promotes the molting process. The molting process has been divided into distinct stages by Drach (1939). Once animals prepare for molting, they are considered to be in premolt (or proecdysis). During premolt, resorption of the old cuticle begins and the epidermis separates from the old exoskeleton (apolysis). The epi- and exo-cuticle layers are then secreted sequentially before the active stage of ecdysis occurs. During ecdysis, animals swallow water until the internal pressure cracks the epimeral sutures along

the carapace. The animal then actively pushes itself out of its old exoskeleton with a series of forceful, coordinated movements (exuviation) (Travis, 1954; Lipcius and Herrnkind, 1982; Phlippen et al., 2000) and continues to draw in water until inflated to a larger, predetermined size. Once ecdysis is complete, the epi- and exo-cuticle layers are calcified and the endocuticle is secreted and calcified. It may take several days for the new cuticle to harden sufficiently. The synthesis of muscle tissue occurs over the course of several weeks and the formation of the membranous layer signals the end of postmolt (metecdysis). The animal then remains in intermolt (anecdysis) until the next molt. The duration of the molt cycle varies significantly, and can last anywhere from a few days for juvenile crabs to more than a year in adults.

Hydrostatic Skeletons

In general, hydrostatic skeletal support systems are found in soft-bodied invertebrates, such as cnidarian polyps and the diverse vermiform animals. They are also found in organs of animals that are otherwise supported by rigid skeletons, such as the muscular hydrostat of the elephant trunk (Smith and Kier, 1989) and the mammalian penis (Kelly, 1999). In rigid skeletal support systems, muscles attach to stiff skeletal elements that provide resistance to the bending, compressive, and torsional forces of muscle contraction. Hydrostatic skeletons, on the other hand, rely on a liquid, such as water, blood, coelomic fluid, or even mesoglea and muscle, to resist the forces of muscle contraction (Chapman, 1958).

Animals with hydrostatic skeletons are typically cylindrical in shape and have a flexible, muscular body wall surrounding a centralized, fluid-filled compartment (Chapman, 1958; Trueman 1975; Gutmann, 1981; Wainwright, 1970, 1982). The body wall typically

includes antagonistic longitudinal and circumferential muscle layers. The fluid is essentially incompressible and transmits pressure in all directions during muscle contraction. Thus, when fibers of one muscle orientation contract, other muscles are stretched and thus serve as antagonists. For example, when a longitudinal muscle contracts in a cylindrical animal with orthogonally arranged musculature, it exerts a compressive force on the animal, causing shortening. Because the enclosed fluid is essentially incompressible, as the animal shortens it increases in diameter. Shortening of the animal can be prevented by resisting an increase in diameter, either by simultaneous contraction of circular muscles or, in animals lacking circumferential muscles, by a tension-resisting cuticle. During longitudinal muscle contraction, resistance to change in diameter results in an increase in pressure within the animal. Changes in shape are, therefore, controlled by the structure and mechanical properties of the cuticle (Harris and Crofton, 1957; Clark and Cowey, 1958).

Research Overview

My general research interests include the biomechanics and evolution of skeletal support systems and the molting process, and how skeletal morphology and molting influence behavioral and ecological adaptations of animals. The goals of this dissertation research are to describe postmolt skeletal support in aquatic and terrestrial crabs in order to understand this important aspect of crustacean growth and how it has influenced their successful invasion of land. This research also investigates how the crustacean body plan can function as both a hydrostat and a rigid skeleton in an attempt to understand the constraints placed on the performance of each system as a result of alternating between the two.

Natural History of Animals

Blue crabs

Blue crabs, *Callinectes sapidus* Rathbun, belong to a family of swimming crabs, the Portunidae. They are entirely aquatic and specialized for swimming; their fifth pair of pereopods is modified as swim paddles. Blue crabs are the target of one of the largest fisheries in the United States.

Blue crabs live in brackish, coastal waters along the Eastern Atlantic coast, from Nova Scotia to Argentina (Williams, 1974). They feed on a broad range of food, even other blue crabs. Mating takes place when the female is soft during her molt to maturity (see Truitt, 1939). The eggs are fertilized and deposited on the abdomen of the female and she then migrates toward the mouth of the bay or estuary where she releases the eggs. The eggs hatch into larvae that drift along the continental shelf where they undergo several molts before becoming juveniles and eventually returning to the estuary where they continue to molt to maturity. Blue crabs molt approximately 20-22 times in their lifetime, with small juvenile crabs molting as frequently as every 7 days and large crabs molting once a year. Male crabs reach a carapace width greater than 160 mm (Williams, 1974).

I chose *C. sapidus* for the research described in this dissertation primarily because it is the only known crab with reliable, visible, external signs of molting (Otwell, 1980). As the new cuticle begins forming beneath the old one, a white line becomes visible along the distal edge of the propodus of the swimming legs (pereopods 5). The coloration of this line changes as the new cuticle continues forming. At approximately 2 days prior to molting, the line is red and the crab is referred to as being in the "peeler" stage. Because of these molt signs, molting can be monitored easily and the exact time of molt can be determined. Furthermore,

since soft-shell crabs are commercially important, peeler crabs are available from local suppliers known as “shedders.”

Blackback land crabs

The blackback land crab, *Gecarcinus lateralis* Freminville, belongs to a family of terrestrial crabs, the Gecarcinidae. They live primarily in tropical and subtropical regions, in particular the Caribbean, the west coast of Central and South America from Mexico to Peru, and the east coast of Central America (Hartnoll, 1988). These crabs burrow in sandy areas at a considerable distance from shore and only return to the ocean to spawn (Hartnoll, 1988). They are omnivorous, feeding primarily on plants but also on carrion (Bliss et al., 1978).

Blackback land crabs have several adaptations for a terrestrial existence. The gills are reduced and their branchial chambers are enlarged, giving a characteristic round appearance, and lined with a respiratory epithelium for obtaining oxygen from the air. They have adapted to low water resources by being nocturnal (Bliss et al., 1978) and drawing water up from the moist sediment using setae along the bases of their fifth pair of pereopods (Bliss, 1963, 1968).

Mating occurs when both sexes have rigid shells (for review of reproduction, see Bliss, 1968, 1979). The female deposits the fertilized eggs on her abdomen and resides in the burrow until they complete development. She then migrates to the ocean where she releases the eggs. The eggs hatch and develop through several larval stages before returning to land as small juvenile crabs. Little is known about the frequency and total number of molts in land crabs, but they reach carapace widths greater than 60 mm.

I chose the blackback land crab for the research described in this dissertation for several reasons. First, this species is highly terrestrial and either molts under protective debris or in burrows that do not reach the water table (Bliss and Mantel, 1968; Bliss et al., 1978). Thus, they molt without the buoyant support of water and, therefore, provide a good comparison to the aquatic blue crab for understanding adaptations to molting. Second, *G. lateralis* is known to inflate their gut with a gas (Bliss, 1968), thereby presenting an interesting biomechanical feature for analysis. Third, the proximity to molt can be estimated by monitoring the regeneration of limb buds (Bliss, 1956; Skinner and Graham, 1972). Finally, this species has been well studied and is easy to maintain in the laboratory.

Topics Investigated

This dissertation is divided into four parts. Chapter II investigates the use of hydrostatic skeletal support during molting in the blue crab, *Callinectes sapidus*. Physiological methods are used to measure and correlate force of muscle contraction and internal hydrostatic pressure in crabs at various postmolt stages. Additional recordings of pressure are made in other regions of the crab body to determine if the body is compartmentalized. This chapter is reproduced with some modification from Taylor and Kier, "Switching Skeletons: Hydrostatic Support in Molting Crabs" SCIENCE 301:209-210 (2003). Modified with permission from AAAS.

Chapter III examines changes in the cuticle mechanical properties at various postmolt stages. A mechanical properties test rig and modified three-point bending apparatuses are used to characterize common materials properties of the cuticle, such as flexural stiffness, Young's modulus of elasticity, and tensile strength.

Chapter IV investigates the use of hydrostatic skeletal support in the blackback land crab, *Gecarcinus lateralis*. Physiological methods are used to measure and correlate force of muscle contraction and internal hydrostatic pressure in crabs at various postmolt stages. Furthermore, the role that the gas-inflated gut plays in providing skeletal support is examined by measuring pressures in the gut and other regions of the body during muscle contraction and by withdrawing the air from the gut. This chapter has been modified from Taylor and Kier (2006) "A pneumo-hydrostatic skeleton in land crabs" NATURE, 440: 1005.

Chapter V explores the effects of scale on the rigid and hydrostatic skeletons in both *C. sapidus* and *G. lateralis*. The principles of how each skeletal support mechanism functions are used to predict how they are influenced by animal size. Morphological measurements are made of the dimensions of a walking limb segment and cuticle thickness, all of which are related to body mass using reduced major axis regression.

Significance

This dissertation research answers two critical and neglected questions in the evolution of animal body plans: how do crustaceans maintain mobility with the loss of their rigid exoskeleton at each molt and how does the arthropod body plan function with hydrostatic skeletal support? Because many arthropods molt, it is likely that this feature is widespread among the arthropods. This research is significant for several reasons:

1. It will broaden our understanding of hydrostatic skeletons by examining its presence in animals with different body plans and locomotor modes. Animals with hydrostatic support are characterized by a cylindrical body plan and orthogonally arranged musculature (Chapman, 1958). Changes in shape are controlled and limited by helically

arranged collagen fibers in the body wall and animals often move by alternating waves of muscle contraction (Trueman, 1975). But arthropods have a point-loaded skeletal system, diverse body shapes, and a large body mass that is held above the substratum by multiple jointed appendages. The coordination of these appendages allows crawling, swimming, and even flying.

2. This research explores the possible role of gases in skeletal support. The use of a gas in combination with a liquid to provide support and facilitate movement is common in engineering, but not in biological systems. Though it is known that insects swallow air into the crop to expand the new cuticle before hardening (Cloudsley-Thompson, 1988; Shafer, 1923), the use of a gas for skeletal support has not been tested. This research will, therefore, describe a new category of hydrostatic skeletal support, a "pneumo-hydrostat."

3. This research will analyze in detail the only known example of an animal that alternates between the two basic skeletal support systems: rigid and hydrostatic. Most animals rely exclusively on only one form or the other. Some animals use the two skeletal support mechanisms for different structures but do not alternate their motor system. For example, bivalve mollusks possess a rigid shell for support but a soft muscular foot and siphon that rely on a hydrostatic mechanism (Chapman and Newell, 1956).

4. This research will provide information on how the alternation between two forms of skeletal support in the Crustacea influences body size. Since postmolt skeletal support has received scant attention, the extent to which it is influenced by scale and thereby influences maximum size in crustaceans is unknown. The few studies of scaling of hydrostatic skeletal support were performed on a single, small, vermiform animal (Quillin, 1998, 1999).

5. A comparison of postmolt skeletal support in aquatic versus terrestrial crabs will reveal differences or similarities that may reflect the divergence of some crustaceans to land or a possible constraint in the evolution of crustaceans. Skeletal support is influenced by different stresses in aquatic and terrestrial environments (Jones, 1978) and how hydrostatic skeletal support in crabs is affected by these differing stresses has consequences for their biology. This information may increase our understanding of some fundamental characteristics that fostered the successful invasion of land by marine organisms.

6. This research will describe a shared characteristic among arthropods and many soft-bodied invertebrates, although the rigid exoskeleton is the defining feature of this group. Thus, this research will examine a feature that may be significant in the evolution of the largest phylum of animals.

More generally, this research will contribute significantly to the overall understanding of how organisms adapt and function according to the physical forces that govern all life. Terrestrial and aquatic environments impose dramatically different forces on animals, and the examination of the skeletal support system in animals that inhabit both environments will provide insight into the effect these forces have on the evolution of fundamental morphological characteristics.

CHAPTER II

HYDROSTATIC SKELETAL SUPPORT DURING MOLTING IN THE AQUATIC BLUE CRAB, *CALLINECTES SAPIDUS* (RATHBUN, 1896)

Summary

Skeletal support systems are essential for support, movement, muscular antagonism, and locomotion. Crustaceans shed their rigid exoskeleton at each molt, yet are still capable of forceful movement. I hypothesize that the soft, water-inflated body of newly molted crabs may rely on a hydrostatic skeleton, similar to that of worms and polyps. I tested this hypothesis by simultaneously measuring the internal hydrostatic pressure and the force exerted during cheliped adduction in crabs at a series of postmolt stages. Additionally, I tested the hypothesis that the crab body is compartmentalized by simultaneously measuring internal hydrostatic pressure in the cheliped and in 5 different locations in the crab body during cheliped adduction. A strong correlation was observed between force and hydrostatic pressure, consistent with hydrostatic skeletal support. Furthermore, the internal hydrostatic pressure in other regions of the body corresponds to the pressure in the cheliped during adduction, indicating that the crab body is not compartmentalized. This is the first evidence of an animal that alternates its motor system between the two basic skeletal types.

Introduction

Animals typically possess one of two general categories of skeletal support throughout their lives: 1. rigid internal or external skeletons (vertebrates, echinoderms, arthropods); 2. soft hydrostatic skeletons (polyps and vermiform animals) (Wainwright, 1982; Wainwright et al., 1982; Alexander, 1983; Schmidt-Nielsen, 1997). Those with rigid skeletons are arranged so that muscles attach to and pull on stiff skeletal elements. The forces of muscle contraction are therefore transmitted by the rigid elements. In contrast, animals with hydrostatic systems are arranged so that the force of muscle contraction is transmitted by an essentially incompressible aqueous fluid (see Chapter 1) (Harris and Crofton, 1957; Clark, 1964; Chapman, 1975, 1958). The fluid typically fills an internal cavity and is surrounded by a flexible container, usually the body wall, which is equipped with muscles and reinforced with connective tissue fibers (Trueman, 1975; Gutmann, 1981; Wainwright, 1982). Muscle contraction increases the pressure in the fluid, causing the deformations or stiffening required for support, movement, muscular antagonism, and locomotion.

During molting, crustaceans become soft and inflated with water, thus resembling an animal with a hydrostatic skeleton. Molting involves secretion of a new cuticle beneath the old one and then shedding of the old, rigid skeleton by uptake of water and cracking the carapace (ecdysis) (see Chapter 1). Through a series of coordinated movements, the molting crustacean extricates itself from the old skeleton (exuviation) (Travis, 1954; Lipcius and Herrnkind, 1982). They then remain in this soft condition for several days before the new cuticle hardens (Drach, 1939; Passano, 1960; Skinner, 1985). During this time, the new cuticle is too soft and flexible to resist the compressive forces exerted by the contraction of muscles. This is often considered to be a period of inactivity (Passano, 1960; Bliss, 1962;

Ryer et al., 1997), but crustaceans are in fact capable of vigorous, rapid, and forceful movement and locomotion immediately following the molt (Olmsted and Baumberger, 1923; Travis, 1954; Lipcius and Herrnkind, 1982; Cromarty et al., 1991; Adams and Caldwell, 1990). The ability of crustaceans to maintain forceful movement and locomotion during molting may be permitted by the use of a hydrostatic skeletal support mechanism.

The possibility of hydrostatic support in crustaceans was mentioned briefly by Drach (1939), Lochhead (1961), and deFur et al. (1985), but has never been tested. I performed a preliminary experiment to determine if the hydrostatic pressure within the animal is necessary to maintain shape and support. First, I cut an opening in the cuticle of a newly molted blue crab, *Callinectes sapidus*, to drain the fluid within. I predicted that once the internal fluid was drained, thus relieving hydrostatic pressure, the animal would lose mobility. Due to a rapid wound repair mechanism, however, there was minimal loss of fluid from the animal. A cheliped of a newly molted crab was then removed at the coxa-basis joint and the internal fluid was allowed to drain. The cheliped collapsed once the fluid had been drained, indicating that without hydrostatic pressure, the cuticle of a newly molted crab cannot maintain shape or support. Though this is not a definitive test, it does offer preliminary evidence consistent with hydrostatic support in crabs.

The switch to a hydrostatic skeleton during molting complicates the function of the arthropod skeleton. The crustacean body plan differs remarkably from that of the typical animal with a hydrostatic skeleton. Common hydrostatic animals, such as nematodes, earthworms, polyps, and sea cucumbers, are all cylindrical in shape with orthogonally arranged musculature (typically circular and longitudinal muscle) and no locomotor appendages. This shape is prominent among soft-bodied animals and is the most economical

shape for maintaining fluids under pressure (Jones, 1978). This vermiform shape limits animals to various forms of peristaltic locomotion. Crustaceans, on the other hand, have muscles arranged as a point-loaded system and they have a range of body shapes that are held above the substrate by a series of articulated locomotor appendages (pereopods). Each pereopod is subdivided into 7 segments, joined together by joints. Crustaceans can walk, run, and swim very efficiently using coordinated movements of the pereopods. The crustacean body plan, therefore, adds complexity to the use of a hydrostat for support and locomotion.

Because fluid transmits pressure in all directions, a consequence of the use of hydrostatic support is that muscle contraction in one body part increases pressure throughout the body (Harris and Crofton, 1957; Chapman, 1958, 1975; Clark, 1964, 1981). Thus, when a crab contracts the *musculus adductor carpopoditis* (Cochran, 1935) to adduct the cheliped, for example, a correlated increase in pressure would be expected in other regions of the body, making it more difficult for other segments and appendages to move independently. Earthworms are able to perform complicated, localized movements because their bodies are divided into multiple compartments (Chapman, 1958). Metamerism such as this not only allows more varied and complex movements, it also prevents the animal from becoming incapacitated if one segment is punctured.

The fact that crabs are able to move their appendages individually and simultaneously suggests that there may be some internal body divisions. It is unclear how the pereopods of crabs could be isolated, since there is no obvious structural mechanism such as a partition. The internal components of the skeleton (endophragm) provide only incomplete compartmentalization. In addition, function of the circulatory system requires that the hemolymph, which is the reservoir for the water taken in at ecdysis, be transmitted to, and

return from, all parts of the body. Transitory isolation of appendages may be possible if expansion of the contracting muscles through the coxae acts as a valve. Earthworms, for instance, can seal off individual segments by contracting sphincter muscles that close the foramina in the incomplete septa (Newell, 1950). Having compartments would facilitate the more complex movements observed in crabs, such as crawling, where several legs move independently.

This chapter describes how crabs maintain body posture and mobility during molting by switching to a hydrostatic skeletal support system.

Materials and Methods

Animals

Male and female "peeler" crabs (within 2-3 days of molt) ranging from 70 - 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 separate recirculating seawater aquaria at a temperature of 19 - 23°C and a salinity of 20 - 35 ppt. Animals were checked every 2 hours for the onset of exuviation. The time postmolt was measured from the time exuviation was complete. To test the hypothesis that crabs switch to hydrostatic skeletal support during molting, individual crabs were measured at three different times postmolt: 1 hour (Soft-shell stage), 12 hours (Paper-shell stage), and 7 days (Hard-shell stage). Each animal was recorded for 5-10 minutes and was returned to its individual tank after the experiment. Only healthy crabs that molted successfully were measured. Of these, only those that survived and appeared healthy after the first experiment were measured in subsequent stages. To test the hypothesis that the crab

body is compartmentalized, individual crabs were measured only during the soft-shell stage (1 hour postmolt). Animals were not fed before experiments.

Pressure and force recordings

Individual crabs, at the postmolt stages described above, were placed in an experimental tank and restrained with Velcro straps on an aluminium slab to prevent movement. Crabs were positioned so that the left cheliped extended laterally and made a 90° angle at the merus-carpus joint. A force transducer (Fort 250, World Precision Instruments, Sarasota, FL, USA) was mounted level with the cheliped of the crab. A low-stretch Spectra cable connected the merus of the extended cheliped to the force transducer. The cable was kept taut to record the force of adduction without movement of the cheliped. A pressure transducer (BLPR, World Precision Instruments, Sarasota, FL, USA) was placed outside the tank at the level of the crab. A catheter of 50-gauge polyethylene tubing and a 23-gauge needle was inserted into the merus of the extended cheliped, into a hemocoelic space just beneath the arthrodial membrane near the merus-carpus joint. The catheter insertion was shallow to avoid entering the underlying muscle tissue.

The theory behind the experimental test is that when the crab contracts the *musculus adductor carpopoditis* (Cochran, 1935) to flex the cheliped towards its mouth (a consistent and stereotyped behavior), it exerts a compressive force on the merus. If support is provided by a hydrostatic mechanism, then this compressive force will be resisted by the constant volume of fluid and tension-resisting cuticle of the merus, causing an increase in hydrostatic pressure.

The force and pressure transducers were connected to a preamplifier, which was connected to a computer via an A/D card. Calibrations of the force and pressure transducers were made before and after each series of experiments. Simultaneous recordings of force and pressure were made at a rate of 65 Hz using data acquisition software (Dataq, Akron, Ohio, USA).

The data were analyzed using Dataq Software. Only clear muscle contraction forces were used for analysis. Instances when the catheter became blocked were not included in the analysis. A blocked catheter was easily recognized by the combination of absence of pressure fluctuation and the absence of increased pressure when the carapace of the animal was depressed manually. Clearing the catheter blockage was accomplished by withdrawing the needle and back-flushing the catheter.

Peripheral pressure recordings

Pressure in peripheral regions of the crab body was measured simultaneously with the internal pressure in the cheliped and the force of cheliped adduction, as described above. Peripheral pressure was measured using a second pressure transducer (BLPR, World Precision Instruments, Sarasota, FL, USA) attached to a catheter of 50-gauge polyethylene tubing and a 23-gauge needle. The catheter was inserted in five different locations in the crab body: the large hemocoelic space at the base of contralateral swimming leg, i.e. pereopod 5, (R5) and the ipsilateral swimming leg (L5), the hemocoelic space just beneath the carapace near the stomach (CA), the base of left pereopod 2 (L2), and the base of the left cheliped (LC). Each crab was measured in all five locations sequentially, though the order in which they were measured was randomized.

To assist with correlating movement of multiple appendages with those of the cheliped, a video camera (Basler A601f, Basler Vision Technologies, Ahrensburg, Germany) was placed above the test aquarium. Pressure and force were recorded using the same preamplifier and A/D card at a rate of 65 Hz. Video was captured using image acquisition software (NI-IMAQ ver. 1.5.2, National Instruments Corp., Austin, TX, USA) and synchronized with pressure and force recordings using LabView 7.1 (National Instruments Corp., Austin, TX, USA). All data were obtained from LabView and analyzed using Dataq Software. Movement of appendages other than the cheliped were noted. Only clear muscle contraction forces of the cheliped alone were used for analysis.

Statistical analysis

Baseline hydrostatic pressure was calculated for each crab and averaged for all animals of each stage. The largest force peak and the corresponding pressure peak were calculated from each crab and averaged for each stage. The data were tested for normality using a Shapiro-Wilcoxon test. Peak force and peak pressure data were not normally distributed for the paper-shell stage ($P < 0.05$) Thus, comparison among soft-, paper-, and hard-shell crabs was made using nonparametric statistics, specifically a Kruskal-Wallis one-way ANOVA and Tukey-Kramer HSD. For peripheral pressure experiments, the baseline and peak hydrostatic pressures in each peripheral location were normally distributed ($P > 0.05$), therefore, the pressure in the cheliped was compared to the pressure in the peripheral location using a paired Student's t-test. All statistical analyses were performed using JMP 5.1 (SAS Institute Inc.).

Results

Force and pressure

Recordings from soft-shell crabs, 1 hour following exuviation, showed significant transient changes in pressure in the merus that are strongly correlated with changes in force (Figure 1). Similar force and pressure records were obtained for all soft-shell crabs measured (n = 15). The magnitude of hydrostatic pressure peaks correlate with the magnitude of force peaks measured ($r^2 = 0.76$, n = 30) (Figure 2).

Recordings from paper-shell crabs, 12 hours following exuviation, also reveal a strong correlation between force and internal pressure (Figure 3). This was consistent among all crabs measured (n = 11).

Recordings from hard-shell crabs, 7 days following exuviation, show no significant changes in pressure corresponding with the large peaks of force (Figure 4). This was consistent among all crabs measured (n = 5).

The mean maximum pressure and force change significantly as the animal progresses from the soft-shell stage to the hard-shell stage (Figure 5). The mean maximum pressure among animals in the soft-shell stage was 1383 Pa (S.D. = 468 Pa, n = 15) and dropped to 150 Pa (S.D. = 116 Pa, n = 5) in the hard-shell stage, with an intermediate value of 812 Pa (S.D. = 251 Pa, n = 11) in the paper-shell stage. The maximum pressures were significantly different between all three stages (Kruskal-Wallis ANOVA, $P < 0.001$; Tukey, $P < 0.05$, n = 15, 11, 5, respectively). The mean maximum force exerted during flexure of the cheliped was 0.09 N (S.D. = 0.03 N) in the soft-shell stage but increased by over an order of magnitude in the hard-shell stage to 0.9 N (S.D. = 0.3 N). Force measurements were significantly different between hard-shell crabs and both soft- and paper-shell crabs (Kruskal-Wallis ANOVA, P <

0.001; Tukey, $P < 0.05$, $n = 15, 14, 5$, respectively), but not between soft- and paper-shell crabs.

Figure 1

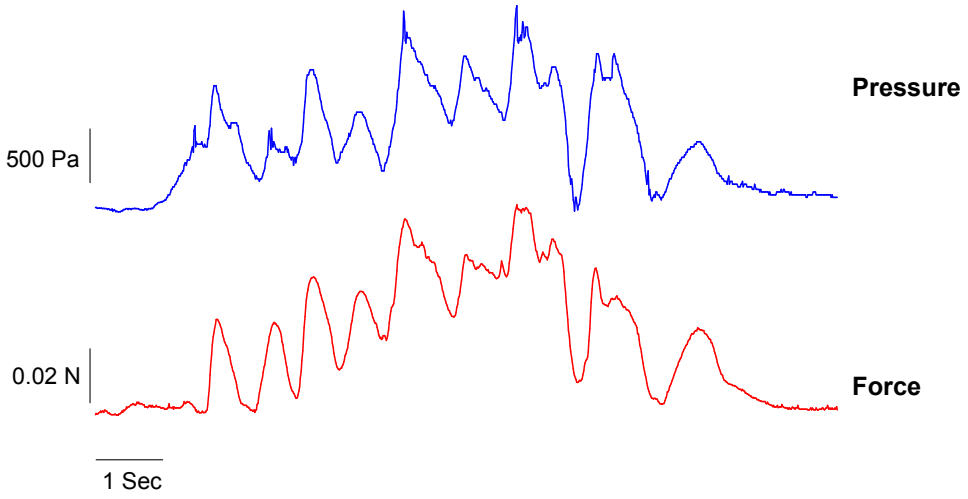

Figure 1. A representative recording of pressure and force from a soft-shell crab 1 hour after exuviation. The upper trace (Blue) is pressure and the lower trace (Red) is force. Peaks of increased pressure correlate with peaks of force. The traces represent approximately 19.5 seconds of recording.

Figure 2

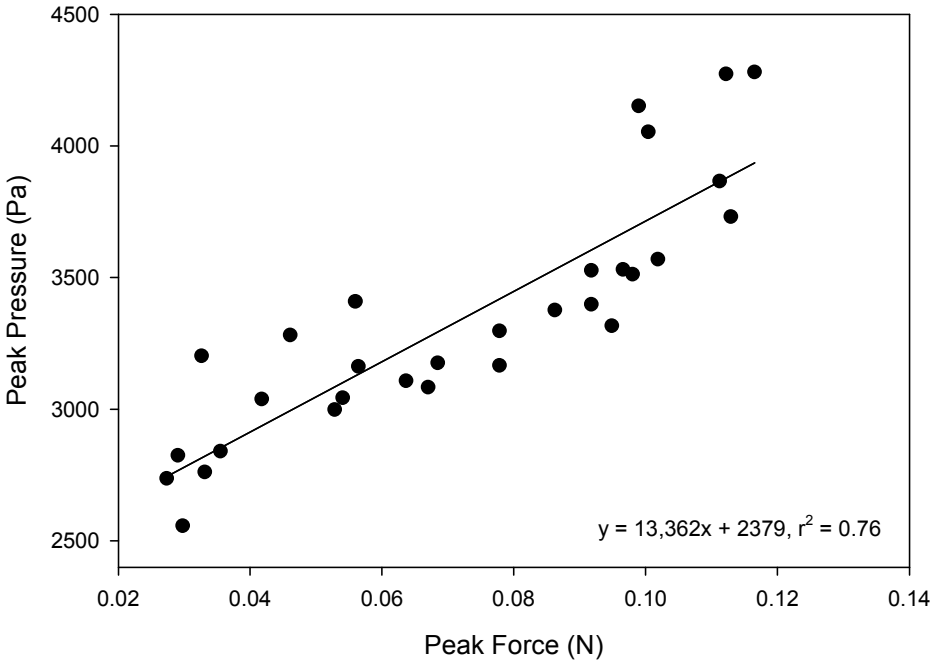

Figure 2. A plot of maximum pressure versus corresponding maximum force in soft-shell crabs. The data were fitted with a least squares regression. The equation for the regression and the r^2 value are listed on the lower right of the plot. The magnitude of pressure peaks correlate with the magnitude of force peaks. n = 30.

Figure 3

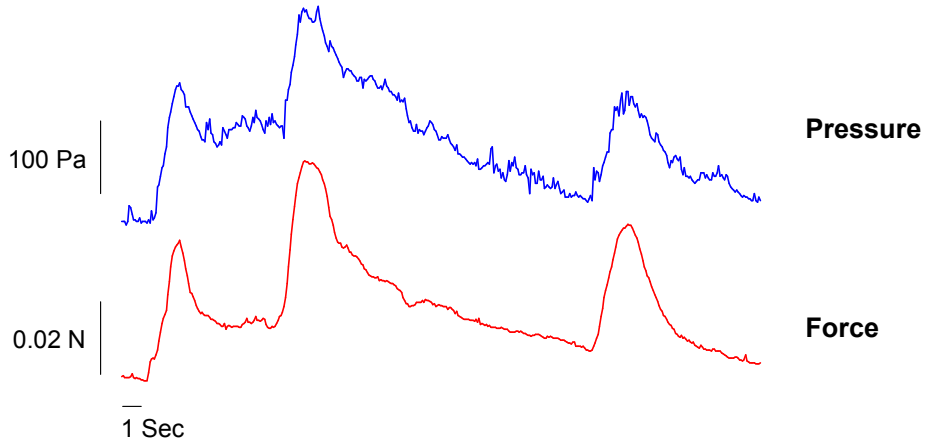

Figure 3. A representative recording of pressure and force from a paper-shell crab 12 hours after exuviation. The upper trace (Blue) is pressure and the lower trace (Red) is force. Peaks of increased pressure correlate with peaks of force. The traces represent approximately 19.5 seconds of recording.

Figure 4

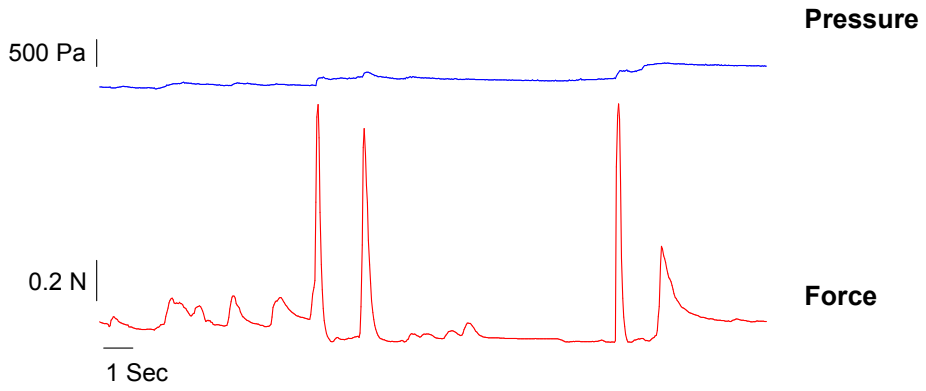

Figure 4. A representative recording of pressure and force from a hard-shell crab 7 days after exuviation. The upper trace (Blue) is pressure and the lower trace (Red) is force. Large forces are present but there are no corresponding increases in pressure. Note that the scale for force in the hard-shell crab is different. The traces represent approximately 19.5 seconds of recording.

Figure 5

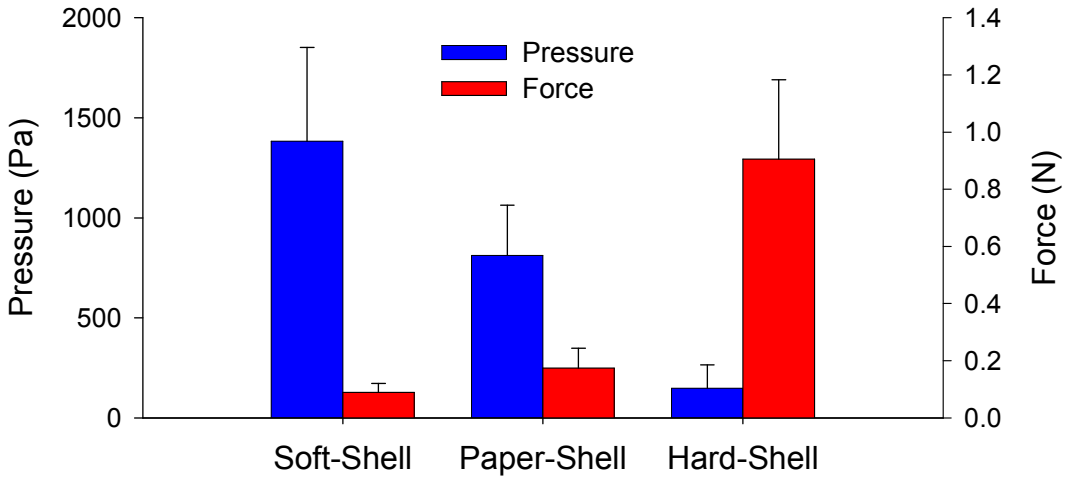

Figure 5. Average maximum pressure and force recorded from postmolt crabs. Maximum pressure and force peaks from individual crabs were averaged. The bars represent overall average for each treatment (soft-shell, paper-shell, hard-shell). There was a significant difference between the pressures measured in all three treatments (ANOVA, $P < 0.001$; Tukey, $P < 0.01$; $n = 15$, 11, and 5, respectively). Force measurements were significantly different between hard-shell crabs and both soft- and paper-shell crabs (ANOVA, $P < 0.001$; Tukey, $P < 0.01$; $n = 15$, 11, and 5, respectively) but not between soft-shell crabs and paper-shell crabs. Error bars represent standard deviation.

Peripheral pressure

Recordings from all five peripheral locations showed strong correlations between the pressure within the cheliped, the pressure at the peripheral location, and force during flexure of the cheliped (Figures 6-10). During each flexure of the cheliped, simultaneous increases in pressure were observed in the cheliped and in each of the peripheral locations.

The average baseline pressures in some of the peripheral locations corresponded to the average baseline pressures in the cheliped (Figure 11). The average baseline pressure in R5 (2041 Pa, S.D. = 503 Pa, n = 19) was not significantly different from the corresponding pressure in the cheliped (2115 Pa, S.D. = 643 Pa, n = 19) (t-test, P = 0.27). This was also the case for L5 (1618 Pa, S.D. = 558, n = 19; Cheliped: 1716 Pa, S.D. = 623 Pa, n = 19) (t-test, P = 0.21). However, the average baseline pressures in the other peripheral locations were significantly less than those in the cheliped: LC (1561 Pa, S.D. = 413 Pa, n = 19; Cheliped: 1750 Pa, S.D. = 443 Pa, n = 19) (t-test, P = 0.005); L2 (1526 Pa, S.D. = 487 Pa, n = 19; Cheliped: 1790 Pa, S.D. = 505 Pa, n = 19) (t-test, P = 0.01); CA (1122 Pa, S.D. = 541 Pa, n = 14; Cheliped:1626 Pa, S.D. = 710, n = 14) (t-test, P = 0.005).

In general, the average maximum peak pressures in the peripheral location corresponded with the average maximum peak pressure in the cheliped (Figure 12). The average peak pressures in R5 (1128 Pa, S.D. = 373 Pa, n = 21) were not significantly different from the corresponding peak pressures within the cheliped (1235 Pa, S.D. = 413, n = 21) (t-test: P = 0.09). This was also the case for all other peripheral locations: L5 (1401 Pa, S.D. = 639 Pa, n = 20; Cheliped: 1145 Pa, S.D. = 560 Pa, n = 20; P = 0.88); CA (796 Pa, S.D. = 236 Pa, n = 16; Cheliped: 786 Pa, S.D. = 179 Pa, n = 16; P = 0.18); LC (795 Pa, S.D. = 178

Pa, n = 18; Cheliped: 827 Pa, S.D. = 125 Pa, n = 18; P = 0.38); L2 (699 Pa, S.D. = 166 Pa, n = 15; Cheliped: 771 Pa, S.D. = 174 Pa, n = 15; P= 0.14).

Figure 6

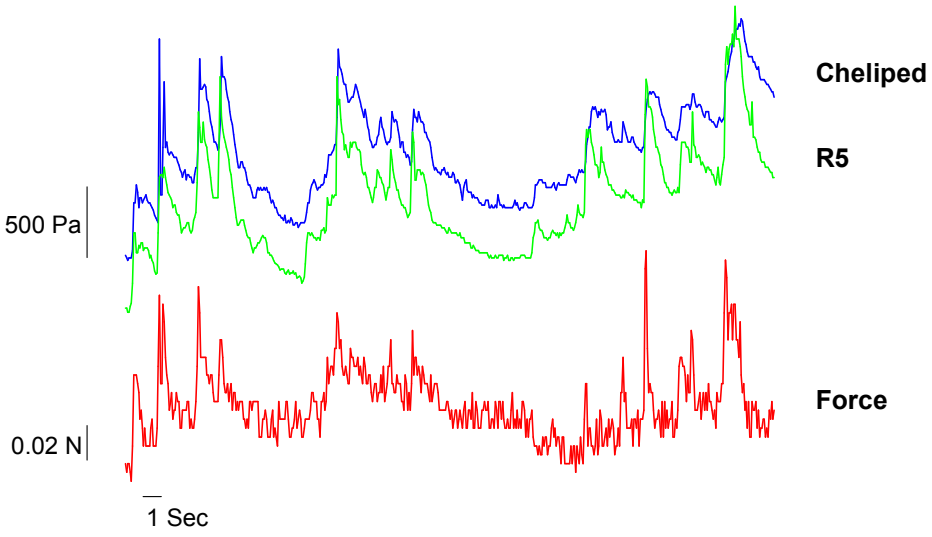

Figure 6. A representative recording of pressure in R5 (base of right fifth pereopod), pressure in the left cheliped, and force from a soft-shell crab 1 hour after exuviation. The upper two traces are pressure (Blue: cheliped, Green: R5) and the lower trace (Red) is force. Peaks of increased pressure occur in the peripheral location and correlate with peaks of pressure in the cheliped and with peaks of force. The traces represent approximately 45 seconds of recording.

Figure 7

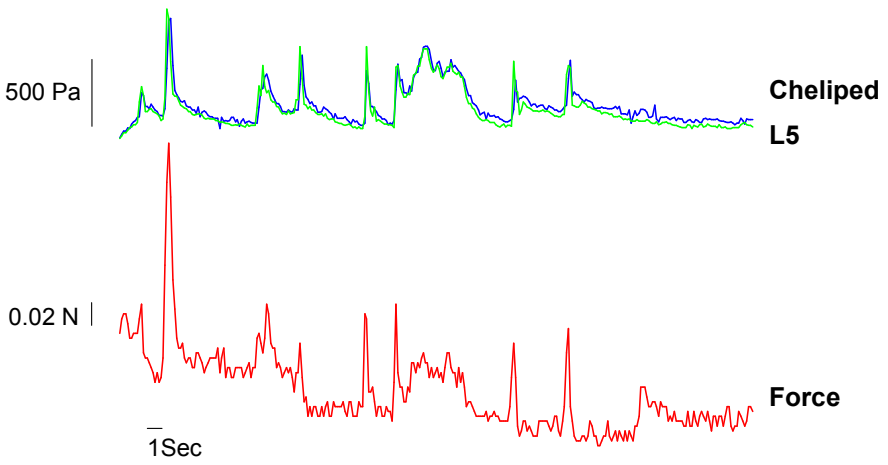

Figure 7. A representative recording of pressure in L5 (base of left fifth pereopod), pressure in the left cheliped, and force from a soft-shell crab 1 hour after exuviation. The upper two traces are pressure (Blue: cheliped, Green: L5) and the lower trace (Red) is force. Peaks of increased pressure occur in the peripheral location and correlate with peaks of pressure in the cheliped and with peaks of force. The traces represent approximately 45 seconds of recording.

Figure 8

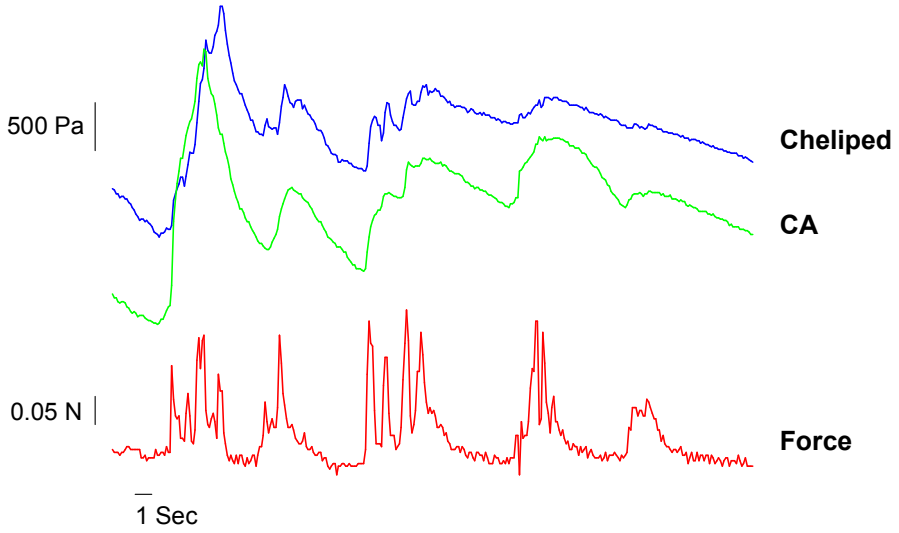

Figure 8. A representative recording of pressure in CA (carapace), pressure in the left cheliped, and force from a soft-shell crab 1 hour after exuviation. The upper two traces are pressure (Blue: cheliped, Green: L5) and the lower trace (Red) is force. Peaks of increased pressure occur in the peripheral location and correlate with peaks of pressure in the cheliped and peaks of force. The traces represent approximately 45 seconds of recording.

Figure 9

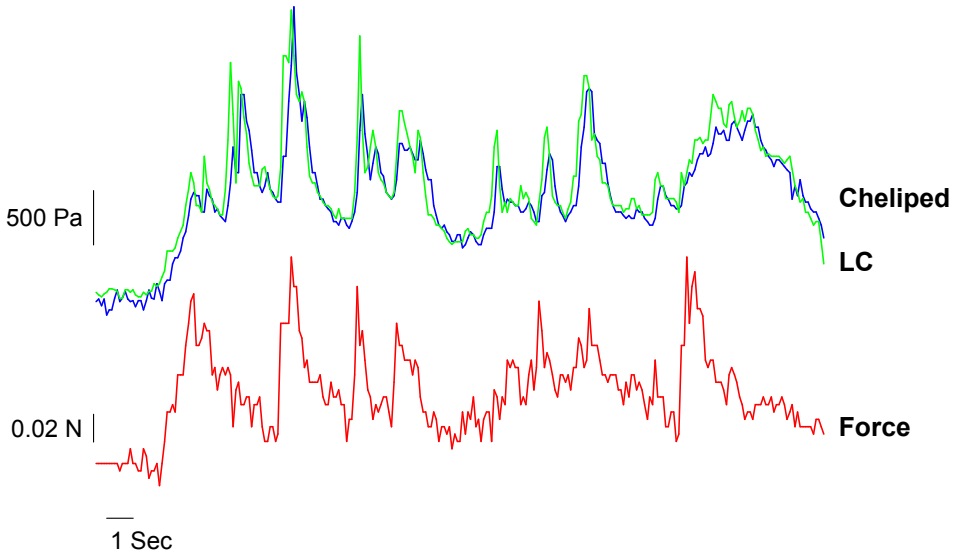

Figure 9. A representative recording of pressure in LC (base of left cheliped), pressure in the left cheliped, and force from a soft-shell crab 1 hour after exuviation. The upper two traces are pressure (Blue: cheliped, Green: L5) and the lower trace (Red) is force. Peaks of increased pressure occur in the peripheral location and correlate with peaks of pressure in the cheliped (at merus-carpus joint) and with peaks of force. The traces represent approximately 25 seconds of recording.

Figure 10

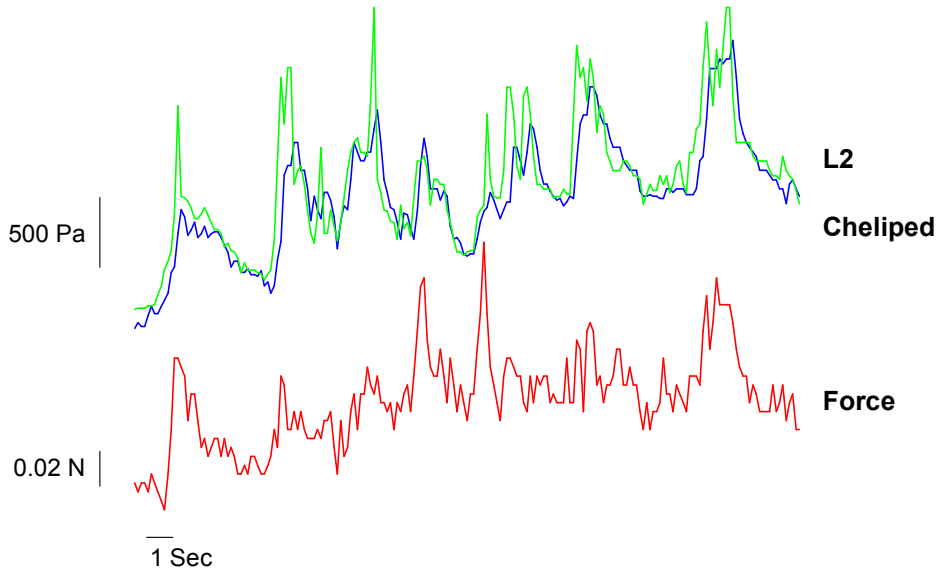

Figure 10. A representative recording of pressure in L2 (base of left second pereopod), pressure in the left cheliped, and force from a soft-shell crab 1 hour after exuviation. The upper two traces are pressure (Blue: cheliped, Green: L5) and the lower trace (Red) is force. Peaks of increased pressure occur in the peripheral location and correlate with peaks of pressure in the cheliped and with peaks of force. The traces represent approximately 25 seconds of recording.

Figure 11

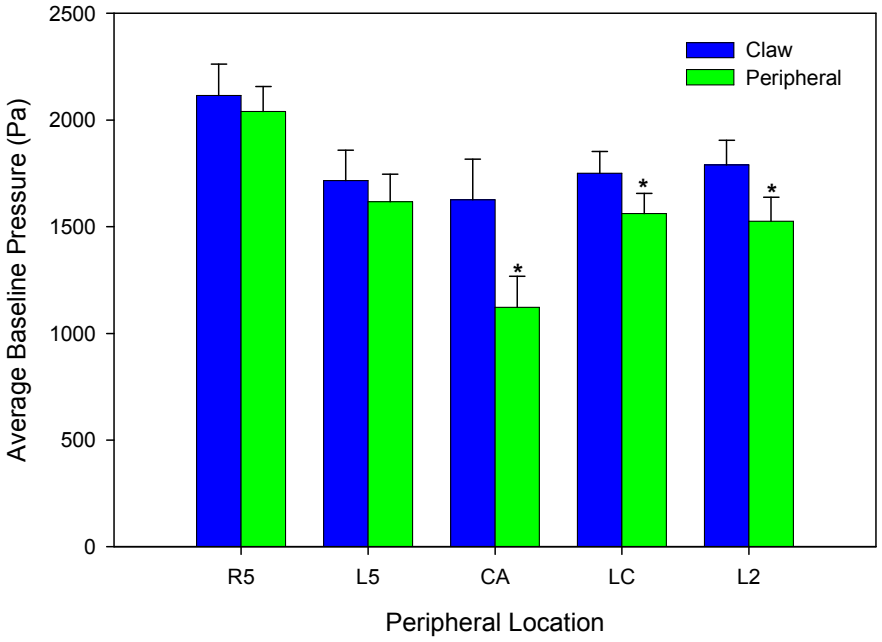

Figure 11. Average baseline pressure in peripheral locations and the cheliped during measurement. Baseline pressure was significantly lower in CA, LC, and L2 than in the cheliped (Student's t-tests, $P < 0.05$) but the baseline pressure in R5 and L5 were not different from that in the cheliped. R5, fifth pereopod on right, L5, fifth pereopod on left, CA, carapace near stomach, LC, left cheliped, L2, second pereopod on left. Error bars = SEM.

Figure 12

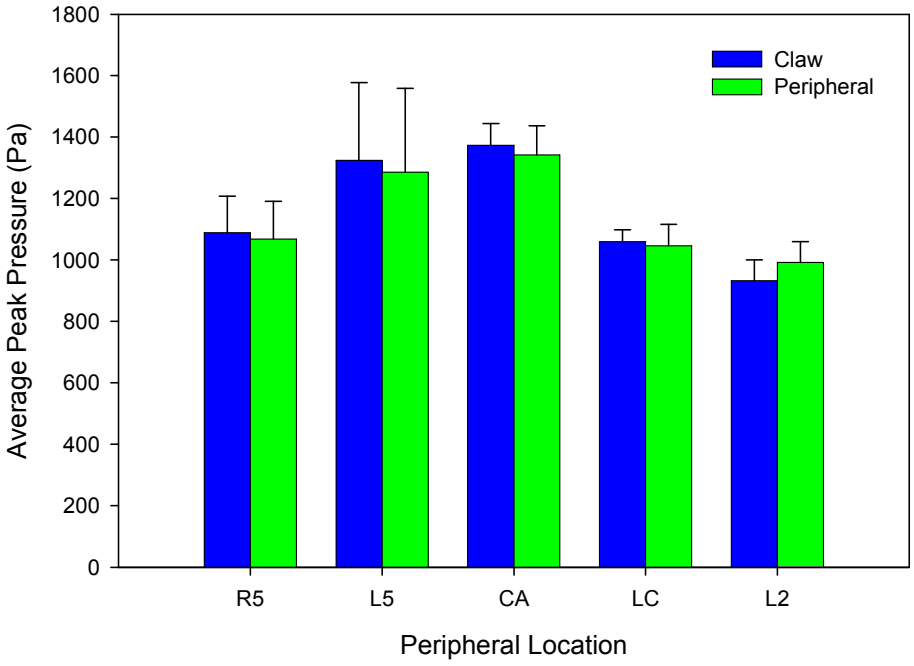

Figure 12. Average peak pressure in peripheral locations and the cheliped during measurement. Peak pressures did not differ between the peripheral location and the cheliped (Student's t-tests, $P > 0.05$). R5, fifth pereopod on right, L5, fifth pereopod on left, CA, carapace near stomach, LC, left cheliped, L2, second pereopod on left. Error bars = SEM.

Discussion

The strong correlation between internal hydrostatic pressure and the force of cheliped adduction is consistent with the use of hydrostatic skeletal support. To generate a moment about the merus-carpus joint, the compressional force on the merus from muscle contraction (which would otherwise shorten this segment) must be resisted. Because the internal fluid is essentially incompressible (liquids generally have high bulk modulus and thus resist volume change), these forces can be resisted if the new cuticle resists tension and thereby resists the increase in circumference that would accompany shortening of the segment (Clark and Cowey, 1958). Thus, increase in internal pressure is predicted to accompany muscle contraction if hydrostatic skeletal support is being used.

Approximately 12 hours after exuviation, crabs enter the "paper-shell" stage during which formation of the cuticle is completed and hardening begins. Although the cuticle is still soft, it is noticeably more rigid than during the soft-shell stage. At this time, there is still a strong correlation between the internal hydrostatic pressure and the force of cheliped adduction. This suggests that at least 12 hours after exuviation, hydrostatic skeletal support is still important for movement and locomotion.

Two to three days are required for the new shell to harden substantially. In the intermolt, hard-shell stage, the cuticle is sufficiently rigid to resist the compressive, bending, and torsional forces of muscle contraction (see Chapter 3). I predicted, therefore, that in the hard-shell state no increase in hydrostatic pressure would be observed during muscle contraction. Indeed, there were large forces associated with cheliped adduction, but I recorded no corresponding peaks of hydrostatic pressure. These data imply that hydrostatic skeletal support is no longer used once crabs have returned to the hard-shell condition.

The gradual shift from hydrostatic to rigid skeletal support means that animals experience significantly different stresses in the various postmolt stages (see Chapters 1 and 6). Thus, an animal in the early postmolt stages must withstand significantly larger pressure-induced stresses throughout its body, with potential implications for both morphology and physiology. Furthermore, the soft, flexible cuticle must have sufficient tensile stiffness to resist deformation by these pressures (see Chapter 3).

While early postmolt animals experience large hydrostatic pressures, the forces they exert are lower than those of intermolt animals. These differences may be a result of muscle atrophy during premolt and regeneration during postmolt, but atrophy is only known to occur in the propodus (Skinner, 1966; Mykles and Skinner, 1981) and muscle regeneration occurs slowly over the course of up to 3 weeks after ecdysis (Skinner, 1966). The muscle contractile properties should be examined throughout the molt cycle to determine the source of these reduced forces. Ultimately, the pressure exerted on the fluid reflects the tension developed by the muscles, and therefore the strength of the animal (Chapman, 1958). The use of a hydrostatic skeleton may in some way limit the force that can be exerted by the animal and may be responsible for the reduced speed and agility of movement observed in postmolt crabs.

The use of hydrostatic support presents a complication for crab locomotion, because the individual segments and pereopods must be compartmentalized in order to function independently. The correlation between peripheral pressure and pressure within the cheliped, however, indicate that the body is not partitioned. When the muscle in the cheliped contracts during flexion, increases in pressure occur throughout the crab body. Though the baseline pressures in some regions of the body, such as the carapace, were slightly lower than the

pressures recorded in the cheliped, peak pressures during muscle contraction were consistent in all regions of the body.

The lower baseline pressures observed beneath the carapace and at the bases of the anterior pereopods may reflect the nature of the circulatory system. In blue crabs, hemolymph is supplied to all of the pereopods by the sternal artery, which carries 87% of the hemolymph, and is returned through long, tube-like sinus channels (Davidson and Taylor, 1995; McGaw and McMahon, 1999; McGaw and Reiber, 2002). The flow of hemolymph to the pereopods can be finely controlled by muscular action within the pereopods and by neurohormonal mechanisms (McGaw and McMahon, 1999). If there is resistance as hemolymph returns through the sinus channels, pressure in the pereopods may be greater than pressures centrally, as observed in this study.

The body of a crab is not compartmentalized yet crabs are still able to move appendages and segments independently of one another. It is currently unclear how this is accomplished. If the animal is fully inflated, then expansion should be observed in other regions of the body. This could be tested by measuring changes in diameter of other appendages. It is also possible that the effective force of other appendages is reduced, since some force is required to resist the increases in pressure. During the experiments, crabs tended to move the backfins rather than the walking legs. The effective force output could, therefore, be tested by measuring pressure and force in the cheliped and backfin, and correlating the measurements with movement.

Despite the significant differences associated with the arthropod body plan, crabs are functioning according to the principles of support of a classical hydrostatic animal.

Crabs in the soft-shell phase are often considered to be immobile and thus little attention has been given to how movement and muscular antagonism is accomplished. When crabs are soft, the claws are less effective for defense and thus, the animals are especially vulnerable to predators, including conspecifics (Reaka, 1975; Ryer et al., 1997). Crustaceans typically spend this vulnerable time in seclusion (Reaka, 1975; Tamm and Cobb, 1978), but they are still quite mobile and active. For instance, the lobster *Homarus americanus* readily performs an escape response while in the soft-shell stage (Cromarty et al., 1991). Some lobsters do not seek shelter, but rather walk about and are capable of intense and coordinated activities (Lipcius and Herrnkind, 1982). Newly molted stomatopods either present a meral spread threat display or flee when their cavities are invaded by conspecifics (Steger and Caldwell, 1983). Olmsted and Baumberger (Olmsted and Baumberger, 1923) observed that soft-shell grapsid crabs move just as actively and rapidly as hard-shell crabs. Newly molted blue crabs readily swim or crawl away from approaching objects (unpublished observation.). Thus, it is clear that the shedding of the rigid exoskeleton does not incapacitate a crustacean.

Although there are a number of examples of individual animals with distinct organs that function with a different skeletal support mechanism, crustaceans represent the only example of an animal that changes the fundamental skeletal support of its motor system from one form to the other repeatedly during life. For example, bivalve mollusks possess a hard shell that protects their internal organs and gives the animal shape, but the burrowing foot and siphons rely on a hydrostatic mechanism (Chapman and Newell, 1956). Elephants have a rigid internal skeleton used for posture and locomotion but their trunks depend on a muscular-hydrostatic mechanism (Smith and Kier, 1989). Likewise, erection in the mammalian penis is maintained by a hydrostatic mechanism (Kelly, 1999). Crustaceans

differ from these examples because the mechanism of skeletal support for the entire body alternates between the two general categories of skeletal support. Because most arthropods grow by molting, it is possible that many undergo this change in skeletal support, including those that live on land without the buoyant support of water.

The ability to alter periodically the fundamental support and motor system of an organism has not been recognized previously. The change from a rigid to a hydrostatic system of support suggests that, in arthropods, the skeletal support system is not as static as typically assumed. It also implies that the arthropod body plan, with numerous jointed appendages arranged in a point-loaded system, can function with hydrostatic skeletal support. Thus, this represents a previously unrecognized characteristic of the largest phylum of animals.

CHAPTER III

MECHANICAL PROPERTIES OF THE RIGID AND HYDROSTATIC SKELETONS OF MOLTING BLUE CRABS, *CALLINECTES SAPIDUS* (RATHBUN, 1896)

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 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 hour (soft-shell stage), 12 hours (paper-shell stage), and 7 days (hard-shell stage) following molting. I measured and compared the flexural stiffness, Young's modulus of elasticity (in tension), and tensile strength for each postmolt stage. I found that the hard-shell cuticle has a flexural stiffness fully 4 orders of magnitude greater than soft-shell and paper-shell cuticle. Although the soft-shell cuticle has a Young's modulus significantly lower than 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.

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 the creation of 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, 2006). Rigid and hydrostatic skeletons differ greatly in their structure and mechanisms of support. Thus, alternation between the two skeletons requires significant structural and functional changes in the cuticle.

Rigid skeletal support systems are common in vertebrates, arthropods, and echinoderms. 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, resulting in compressional, torsional, and bending forces (Wainwright, 1982) that must be resisted by the rigid elements in order to avoid failure. In crabs, the body is typically held above the substrate 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 LaBarbera, 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 LaBarbera, 1993). Rigid skeletons thus function primarily by resisting compressional, bending, and torsional forces.

Hydrostatic skeletons are common in soft-bodied invertebrates including polyps, such as sea anemones, and many types of worms. Classical hydrostatic skeletons have no rigid elements, are typically cylindrical in shape, and consist of a flexible, muscular body wall surrounding a fluid-filled cavity (Chapman, 1958; Trueman 1975; Gutmann, 1981; Wainwright, 1970, 1982). 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 be able to resist this tension (Wainwright, 1970). The structure and mechanical properties of the body wall can therefore control shape changes of the animal (Harris and Crofton, 1957; Clark and Cowey, 1958). Hydrostatic skeletons thereby function primarily by resisting tensile forces.

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 take in 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, much like other animals with hydrostatic skeletons. The muscular arrangement, however, remains unchanged.

Concurrent with this dramatic change in the mechanical function of the cuticle are the remarkable changes that occur in its structure throughout the molt cycle (Roer and Dillaman, 1993). The cuticle changes from a rigid material that requires significant force to 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 takes place, 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 completely 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, 1963, 1965). At approximately 12 hours after ecdysis the cuticle attains the texture of paper due to the start of tanning and mineralization. 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 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 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,b; 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). In this study, I document the changes in the mechanical properties of the cuticle associated with its change from a rigid to a hydrostatic skeleton. I 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. I 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*, was used 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 at a temperature of 19°C and a salinity of 15 to 20 ppt (Instant Ocean Artifical Seawater, Aquarium Systems Inc, Mentor, OH, USA). Animals were checked every 2 hours 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 hour (soft-shell stage), 12 hours (paper-shell stage), and 7 days (hard-shell stage). The animals were not fed before or during experiments.

Cuticle Samples

Both chelipeds were removed from crabs at the three specified postmolt stages. One cheliped was used for tensile testing while the other was used for bending tests. If the chelipeds differed in size, the larger one was used for tension tests (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 tests to be performed in the proper orientation for loading. 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 using 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 sea water 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 sea water 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 #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 (Figure 13). The insect pins were attached to the side of a solid brass bar (100 x 19 x 19 mm) so that they extended vertically, 10.19 mm apart. Cuticle samples were rested 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 x 11 x 3 cm) filled with sea water on the base of a dissecting microscope. Two force transducers (AE-801 Sensor Element, SensorOne Technologies Corp., Sausalito, CA, USA) were used. A #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 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 then securely attached to a 3-axis micromanipulator and 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 were recorded. The total displacement was less than 10% of the sample length.

The force transducers were connected to a preamplifier 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 made at a rate of 488 Hz using data acquisition software (WinDaq/Lite, Dataq Instruments, Akron, OH, USA). The data were analyzed using Dataq Software.

Calibrations of the force transducers were made before and after each series of experiments with objects of known weights. 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 mm, 0.02 mm, 0.025 mm, 0.04 mm, and 0.05 mm.

Hard Cuticle Apparatus

A tensometer (Hounsfield Tensometer, Tensometer Limited, Croydon, England) was adapted for use in a 3-point bending test (Figure 14). 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) which was 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 90º 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

Figure 13

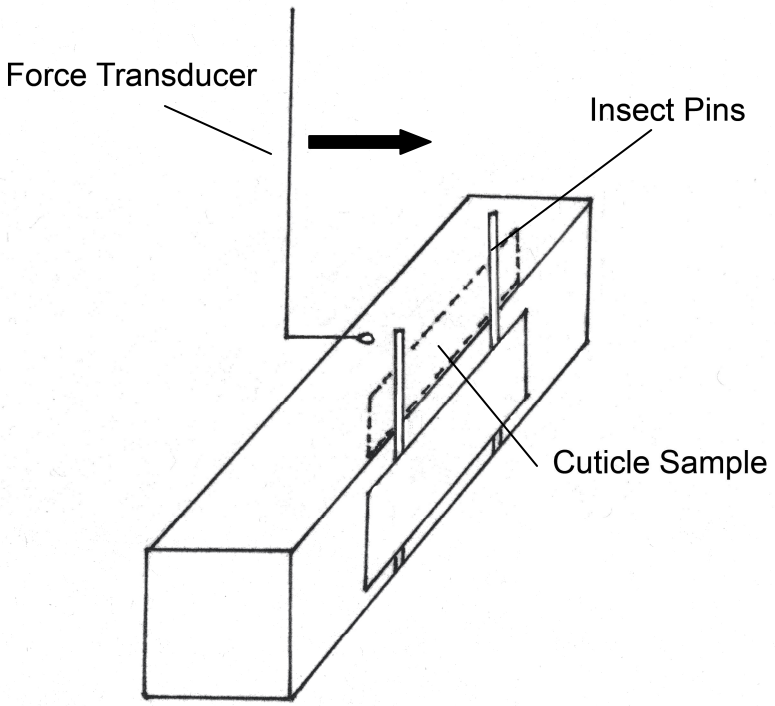

Figure 13. Diagram of bending apparatus used for soft- and paper-shell cuticle samples. Block arrow indicates direction of movement.

Figure 14

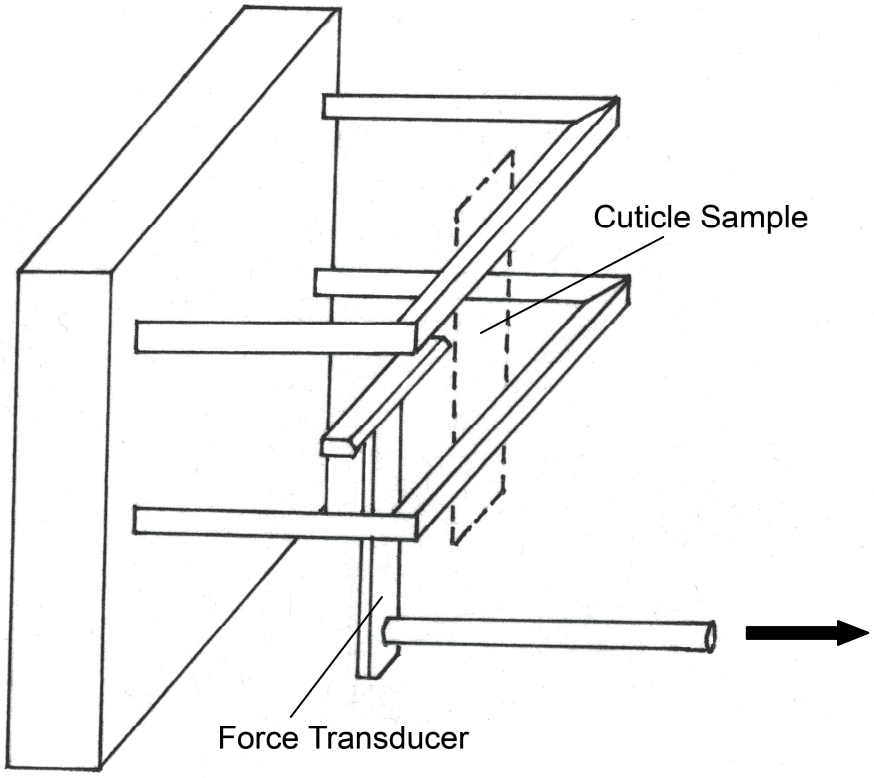

Figure 14. Diagram of bending apparatus used for hard-shell cuticle samples. Block arrow indicates direction of movement.

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 in the soft and paper cuticle but with a range of heavier weights and with plastic shim stock of the following thicknesses: 0.25 mm, 0.31 mm, 0.4 mm, 0.5 mm, 0.6 mm, 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). Both ends of the cuticle samples were sandwiched between two 19 mm x 19 mm square aluminum grips using cyanoacrylate to aid the attachment. Soft and paper cuticle samples were placed in 0.90 mm thick aluminum 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 and data were acquired using the stock Lloyd software.

Analysis

Bending

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

$$y = \frac{Fl}{48EI}$$

where y is the deflection of the sample, F is the force applied, 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). The 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 nonparametric analyses in Zar (1999).

Tension

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

$$\sigma = \frac{F}{A}$$

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 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 average thickness of soft cuticle was 20 μm (S.D. 2.9 μm, n = 9) and paper cuticle was 30 μm (S.D. = 13 μm, n = 15).

Strain, ε, was calculated as engineering strain,

$$\varepsilon = \frac{\Delta L}{L_0}$$

where ΔL is the change in length of the sample and L_0 is the initial sample length.

A stress-strain plot was created for each cuticle sample, from which the Young's modulus of elasticity (stiffness) and the tensile breaking strength were obtained. The Young's modulus of the material in tension is the ratio of stress to strain or the slope of the plot. For non-Hookean materials (those that do not show a linear relationship between stress and strain), the Young's modulus can be estimated as a tangent modulus (Hepburn and Joffe, 1974). This was measured as the slope of the linear portion of the stress-strain plot between 10-50% of the strain at failure. The tensile strength of a material is the stress at 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 were

determined to have a normal distribution by the Shapiro-Wilcoxen test, so an ANCOVA was used to determine differences among the cuticle samples with storage time as a covariate.

Results

Bending

Soft, paper, and hard cuticle samples have a flexural stiffness of 2.0×10^{-9} Nm^2 (S.D. = 2.3×10^{-9} Nm^2, n = 10), 7.2×10^{-9} Nm^2 (S.D. = 8.6×10^{-9} Nm^2, n = 10), and 1.8×10^{-5} Nm^2 (S.D. = 2.0×10^{-5} Nm^2, n = 12), respectively (Figure 15). Thus, the hard cuticle has a flexural stiffness that is four orders of magnitude greater than both paper and soft cuticle (Kruskal-Wallis, $X^2 = 13.8$, $P < 0.001$; $Q = 2.39$, $P < 0.05$; n = 12,10, 10). The measured flexural stiffness of soft and paper cuticle samples are not statistically different.

Figure 15

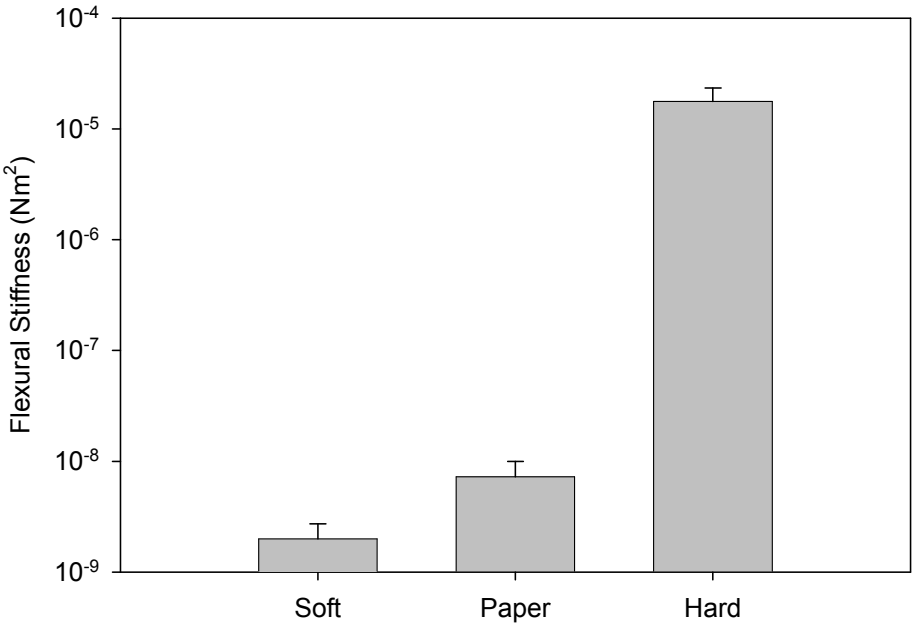

Figure 15. Average flexural stiffness, *EI*, of soft, paper, and hard cuticle. (n = 10, 10, 12, respectively). Error bars = SEM.

Figure 16

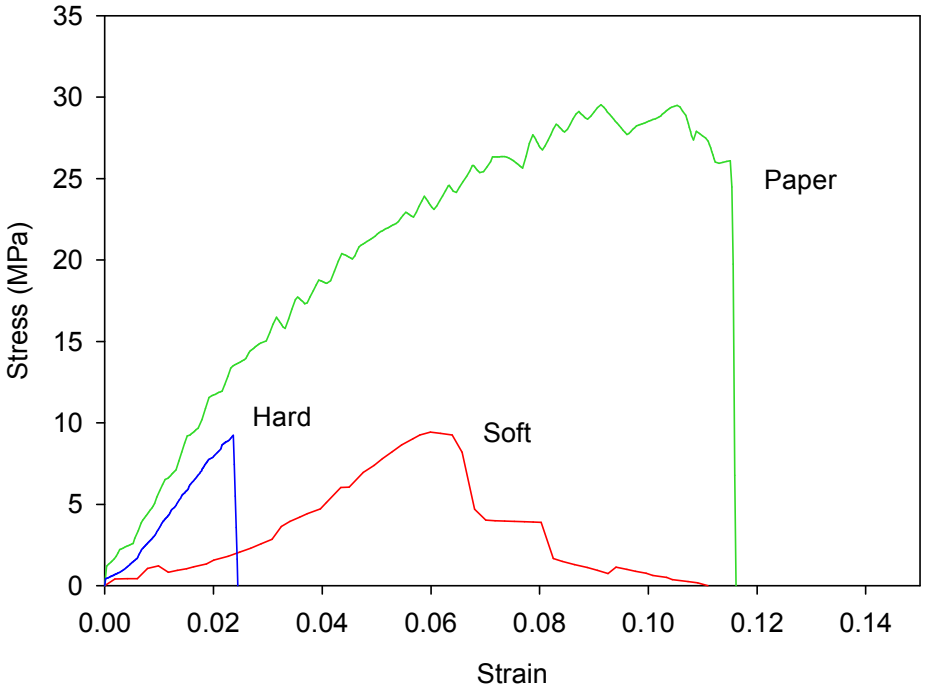

Figure 16. A typical stress-strain plot for soft, paper, and hard cuticle.

Tension

The stress-strain plots for soft, paper, and hard cuticle all show an approximately linear portion and a distinct point at failure (Figure 16). The Young's modulus of the soft cuticle samples was 137 MPa (S.D. = 91 MPa, n = 15), which was significantly lower than paper-stage cuticle, 274 MPa (S.D. = 186 MPa, n = 18), and hard cuticle, 318 MPa (S.D. = 133 MPa, n = 12) (ANCOVA, $P < 0.05$; Tukey, $P < 0.05$; n = 12) (Figure 17). 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 tensile strengths of 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 (Figure 18). The tensile strength of paper cuticle appears to be greater than 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 strength of the cuticle samples (ANCOVA, $P < 0.05$).

Figure 17

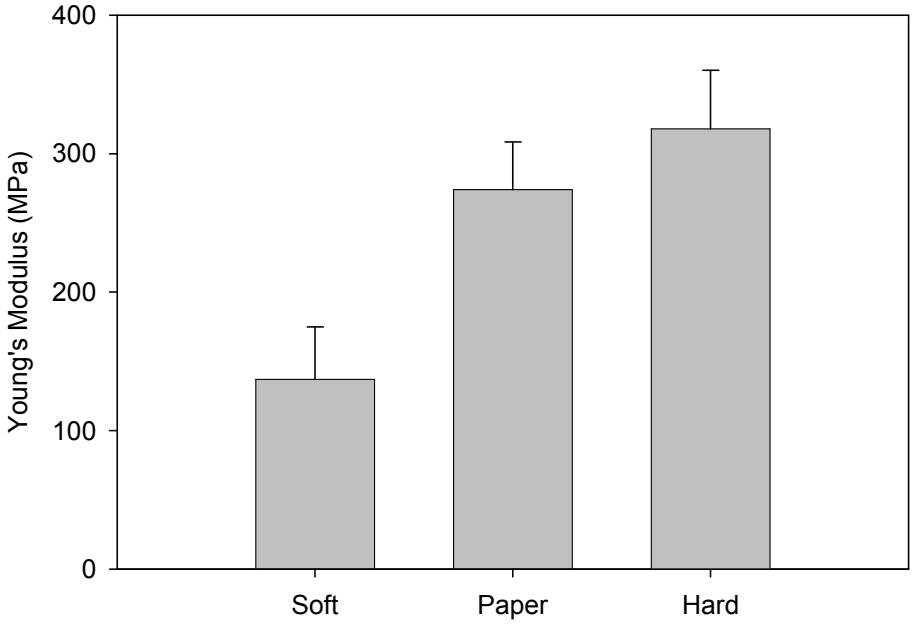

Figure 17. Average Young's moduli, *E*, of soft, paper, and hard cuticle. (n = 15, 18, 12, respectively). Error bars = SEM.

Figure 18

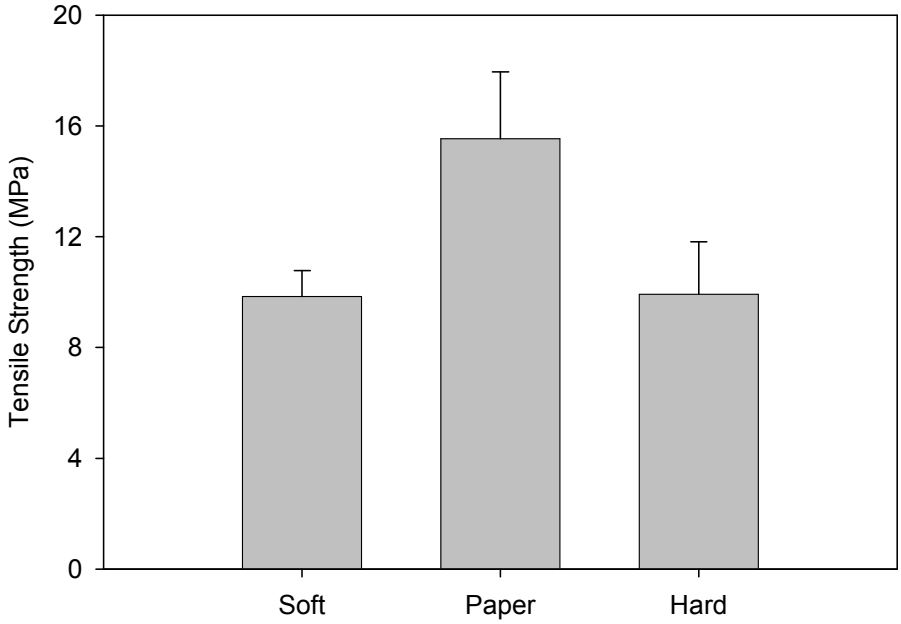

Figure 18. Average tensile strength of soft, paper, and hard cuticle. (n = 15, 19, 12, respectively). Error bars = SEM.

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. For crabs to alternately function with each type of skeleton, the cuticle must be able to withstand compressional, bending, and torsional forces during intermolt periods and tensile forces during molting. Although the cuticle during the soft shell stage is completely inadequate in supporting compressional and bending forces, our results demonstrate that it can bear the same magnitude of tensile stresses as the much more robust intermolt cuticle.

For the first twelve hours following ecdysis, the soft cuticle has a remarkably low flexural stiffness ($2 - 7 \times 10^{-9}$ Nm^2). This is not surprising because soft cuticle folds so easily when handled that it obviously cannot resist any significant bending forces. This presents a fundamental problem for functioning as a typical rigid exoskeleton because movement and muscular antagonism requires that the cuticle resist the bending forces associated with muscle contraction. The claw muscles of decapod crustaceans are capable of producing large stresses, as high as 400 to 2000 kNm^{-2} during intermolt (Josephson, 1993). During molting, intrinsic or extrinsic factors may reduce force production of some muscles in order to prevent damage to muscle fibers or cuticle. In fact, the claw muscles undergo significant atrophy prior to molting (Skinner, 1966; Mykles and Skinner, 1981, 1982, 1990), but 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 (pers. observ.) 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 twelve hours 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} Nm^2). This large increase in flexural stiffness corresponds to the progression of the mineralization process (Vigh and Dendinger, 1982; Dendinger and Alterman, 1983). By seven 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 is once again functioning with a rigid skeletal support mechanism (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 soft cuticle within an hour of exuviation is only 132 MPa, but increases significantly to 379 MPa 12 hours later during the paper stage and stabilizes at 361 MPa a week later during the hard stage. Though there are no known previously reported values of tensile stiffness for newly molted animals, the tensile stiffness for hard cuticle is similar to that reported for the crab *Scylla serrata* (481 MPa) (Hepburn et al., 1975) and the prawn *Panaeus mondon* (461-549 MPa) (Joffe et al., 1975b). Furthermore, a similar pattern of a rapid increase in flexural stiffness during the first 12 hours 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 is resisted as the stiffness of the cuticle increases. The increase in Young's modulus observed in paper stage cuticle is likely 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 hours (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 hard cuticle do not afford it any greater tensile strength than soft cuticle. 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), but are similar to previous measurements of blue crabs (5.6-15 MPa) (Dendinger and Alterman, 1983). These reported values of tensile strength are in the same range as ours despite the fact that they were taken from the carapace and frozen before testing.

Previous studies on blue crabs have shown an increase in carapace tensile strength immediately after molting (Richards, 1951; Dendinger and Alterman, 1983) followed by a significant decrease by 24 hours postmolt (Dendinger and Alterman, 1983). This same pattern was also observed in locust cuticle (Hepburn and Joffe, 1974). Our data show a similar pattern, with tensile strength higher in paper cuticle than soft and hard cuticle, but this difference was not statistically significant, perhaps because of rather high variance in the

paper cuticle samples. Some cuticle samples were noticeably more similar to the soft-shell stage at the 12 hour point, suggesting that some individuals have slightly different rates of hardening. Additionally, our limited access to the materials testing apparatus meant that some samples had to be stored (refrigerated in seawater) for up to two weeks before testing and thus may have been degraded.

The remarkable similarity in tensile strength between the soft cuticle and the hard cuticle is of interest in the context of the functional and structural changes in the cuticle during the molt cycle. During the soft-shell and paper-shell stages, the cuticle must provide resistance to tensile forces generated by pressurization of the hydrostatic skeleton. A relatively high tensile strength is necessary to prevent rupture of the cuticle and loss of hydrostatic pressure, immobilizing the animal. Additionally, loss of hydrostatic pressure may be limited by the rapid arthropod hemolymph clotting mechanism, which aids in wound repair and immune defense (Theopold et al., 2004). The paper cuticle may achieve a higher tensile strength than soft cuticle, as reported in previous studies, because of the tanning and calcification process. Calcification begins slowly after ecdysis, but starts to increase significantly between 10 and 24 hours after ecdysis (Vigh and Dendinger, 1982). The calcification process begins in the interprismatic septa of the exocuticle which creates a honeycomb-like structure that gives some rigidity with a small amount of calcium deposition (Compère et al., 1993; Dillaman et al., 2005). The initial deposition is amorphous calcium carbonate which may be stronger because of its isotropic structure (Dillaman et al., 2005). Thus, the paper cuticle may have increased tensile stiffness and strength without being rigid. As the mineralization process continues, the amorphous calcium carbonite is transformed into crystals of calcite, making the cuticle more brittle (Joffe et al., 1975b; Dillaman et al.,

2005) and thus reducing the tensile strength. The tensile strength of hard cuticle is nevertheless significant and is important for its function in resisting bending forces since they induce both tensile and compressive stresses in the material.

The observed differences in mechanical properties may also be partially attributable to differences in the water content of the cuticle. Preliminary Thermogravimetric analysis of *C. sapidus* cuticle reveals that the soft cuticle contains approximately 30% more water than hard cuticle (J. R. A. Taylor, unpublished data). Water acts like a plasticizer, imparting greater softness and flexibility to a material (Levine and Slade, 1988). Therefore, the extra water in the soft cuticle may be partially responsible for differences in the observed Young's modulus.

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 in 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). These tensile properties provide the tensile support needed for hydrostatic skeletal support.

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 two-fold 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 slight differences in cuticle associated 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. Despite these differences, the material properties of some insects and crustaceans are reportedly similar (Joffe et al., 1975b). A more extensive study of the mechanical properties in other groups of arthropods and other molting phyla may provide broad-ranging new insights into the mechanics of the molting process and reveal additional important characteristics relevant to the evolution of ecdysozoans.

CHAPTER IV

PNEUMO-HYDROSTATIC SKELETAL SUPPORT DURING MOLTING IN THE BLACKBACK LAND CRAB, *GECARCINUS LATERALIS* (FREMINVILLE 1835)

Summary

There are two general categories of skeletal support in animals: rigid and hydrostatic. Rigid endo- and exo-skeletons rely on stiff elements to transmit the forces of muscle contraction, while hydrostatic skeletons rely on an incompressible fluid, such as water, hemolymph, or even muscle. No other type of skeletal support system has been described in animals. In this chapter, I show that the land crab, *Gecarcinus lateralis*, uses an unconventional type of hydrostatic skeleton that includes both gas and liquid, a pneumo-hydrostat, each time the exoskeleton is shed during molting. I simultaneously measured the hydrostatic pressure within the cheliped, the gas pressure within the gut, and the force exerted as the cheliped flexed. A strong correlation was observed between force and pressure, consistent with the use of hydrostatic skeletal support. Furthermore, the pressures within the cheliped and gut changes simultaneously and were not significantly different. When air in the gut was withdrawn, pressures in the gut and cheliped decreased simultaneously. This suggests that the gas in the gut is responsible for increased body turgor enabling movement and locomotion. This is the first experimental evidence of a locomotor skeleton that uses a gas, thereby establishing a new category of hydrostatic skeletal support.

Introduction

As crabs evolved adaptations for life on land, they retained a molting process similar to that of aquatic crabs and other arthropods; they secrete a new cuticle beneath the old, shed the old skeleton (exuviation), inflate to a larger size, and subsequently harden the new skeleton (Drach, 1939; Passano, 1960; Skinner, 1985) (see Chapter 1). But molting in air presents additional challenges because there is little water available for inflation and the air does not provide buoyancy to help support the animal until the new cuticle hardens. The blackback land crab, *Gecarcinus lateralis*, solves this problem by supplementing water with air. This species absorbs some moisture from the sediment using setae on the legs (Bliss, 1956, 1968, 1979; Bliss et al., 1966) and also inflates the foregut with a gas (Bliss, 1956, 1968, 1979). Dissection of newly molted animals reveals the foregut to be greatly distended, which produces the prominent gastric arch in the exoskeleton (Bliss, 1956, 1968, 1979). The release of gas can be heard when the gut is punctured with a needle and many air bubbles are visible inside the foregut (J. R. A. Taylor, unpublished observation). Although little is known about the gas, it is likely air that is swallowed during ecdysis, a process common among insects (Reynolds, 1980; Miles and Booker, 1998). The water and air used to inflate the animal are hypothesized to provide skeletal support following molting.

Newly molted crabs remain soft for several days before the new skeleton hardens sufficiently to support the forces of muscle contraction. Nevertheless, crabs are not incapacitated during this time. Large, newly molted land crabs drag their bodies across the substrate using their hefty claws, while small crabs easily crawl holding their bodies above the substrate (J. R. A. Taylor, unpublished observation). In the aquatic blue crab, *Callinectes sapidus,* mobility is maintained following molting by switching to a hydrostatic skeleton

(Taylor and Kier, 2003; Chapter 2). Hydrostatic skeletons are common in soft-bodied animals and are arranged so that the force of muscle contraction is transmitted by an essentially incompressible aqueous fluid (Chapman, 1958, 1975; Clark, 1964; Harris and Crofton, 1957) (see Chapter 1). Animals with hydrostatic skeletons are typically cylindrical in shape and have a flexible, muscular body wall reinforced with connective tissue fibers, surrounding a fluid-filled compartment (Trueman, 1975; Gutmann, 1981; Wainwright, 1982). Muscle contraction increases the pressure in the fluid, causing the deformations or stiffening required for support, movement, and locomotion.

It is more challenging to support a crab hydrostatically on land than in water, because there is a greater risk of water loss and there are larger gravitational forces. This may be why most terrestrial hydrostats are relatively small (e.g., annelids, insect larvae, gastropods, nematodes, platyhelminthes, and nemertines). For crabs, inflating the gut with air rather than water may also reduce the amount of weight newly molted animals must support. In general, greater pressures are required to support terrestrial hydrostats than aquatic ones (Jones, 1978).

In this chapter, I describe a series of experiments designed to test for the use of pneumo-hydrostatic skeletal support in *Gecarcinus lateralis*. First, I tested for hydrostatic skeletal support by simultaneously measuring the internal hydrostatic pressure and force of muscle contraction within the cheliped of newly molted crabs. I then examined the role of gut pressure in postmolt skeletal support by measuring the internal pressure of the cheliped and the gut simultaneously during cheliped flexure. To explore further the role of the gas in providing body turgor, I performed an additional experiment in which I relieved the pressure within the gut.

Materials and Methods

Animals

The blackback land crab, *Gecarcinus lateralis*, was selected for this study because it is highly terrestrial; it only returns to water to spawn and it lives in burrows that do not reach the water table (Bliss and Mantel, 1968). Approximately 150 land crabs were captured by hand in the Fajardo Reserve, Fajardo, Puerto Rico in June 2003 and shipped to the University of North Carolina at Chapel Hill. All crabs were kept in environmental chambers at 27°C on a 12 h light:12 h dark cycle. Humidifiers were used to keep room humidity between 60-90%. Individual crabs were maintained in separate, plastic, shoebox-size containers with moistened sand and dishes of water. Containers were cleaned and crabs were fed carrots and lettuce twice a week. The sand was changed regularly.

To induce molting, 6 limbs were removed from each crab by injecting a small amount of distilled water at the base of each leg (Bliss and Mantel, 1968). The proximity to molt was determined by monitoring the growth of limb regenerates (Bliss and Mantel, 1968; Skinner and Graham, 1972). The time postmolt was calculated from the time exuviation was complete, or if exuviation was not observed, was estimated based on time of previous check and softness of the crab. Individual crabs were measured twice postmolt, within 12 hours and at approximately 7 days if the crabs were healthy and ate their exuvium. Some measurements were made on hard crabs at longer times postmolt. Each animal was recorded for 5-10 minutes and was returned to its container after the experiment.

Pressure and force recordings

Individual crabs were restrained with Velcro straps on an aluminium slab to prevent movement. Crabs were positioned so that the left cheliped extended laterally and made a 90° angle at the merus-carpus joint. A force transducer (Fort 250, World Precision Instruments, Sarasota, FL, USA) was mounted level with the cheliped of the crab. A low-stretch Spectra cable connected the merus of the extended cheliped to the force transducer. The cable was kept taut to record the force of adduction without movement of the cheliped. A pressure transducer (BLPR, World Precision Instruments, Sarasota, FL, USA) was placed at the level of the crab. A catheter of 50-gauge polyethylene tubing and a 23-gauge needle was inserted into the merus of the extended cheliped, into a hemocoelic space just beneath the arthrodial membrane near the merus-carpus joint. The catheter insertion was shallow to avoid entering the underlying muscle tissue.

Gut pressure

Gut pressure measurements were made on newly molted crabs within 12 hours postmolt. The same apparatus was used, only a second pressure transducer was used to measure pressure within the gut (BLPR, World Precision Instruments, Sarasota, FL, USA). The second pressure transducer catheter was inserted dorsally through the carapace, anterior and slightly left of the midline above the foregut. The needle was inserted just shallow enough to pierce the cuticle and lining of the gut. Dissections afterwards confirmed the location of the needle based on the puncture mark. Simultaneous recordings were made of force from cheliped flexure and the pressures within the cheliped and the gut.

Gas removal

Gas removal experiments were performed on newly molted crabs within 12 hours postmolt. Two pressure transducer catheters were used. One was inserted into the cheliped and the other was inserted into the gut, as described above. After both catheters were successfully inserted and a baseline pressure was recorded, a syringe with a 23-gauge needle was inserted into the gut on the right side, opposite the pressure transducer catheter. Air was withdrawn from the gut using the syringe for approximately 1-2 minutes.

Data recording

Signals from the force and pressure transducer preamplifiers were fed to an analog to digital conversion unit (DI-700, Dataq Instruments, Akron, OH, USA) connected to a microcomputer. Calibrations of the force and pressure transducers were made before and after a series of experiments. Simultaneous recordings of force and pressure were made at a rate of 488 Hz using data acquisition software (WinDaq/Lite, Dataq Instruments, Akron, OH, USA).

Data were analyzed using Dataq Software. Only clear muscle contraction forces were used for the analysis of pressure and force. Instances when the catheter became blocked were not included in the analysis. A blocked catheter was easily recognized by the combination of absence of pressure fluctuation and the absence of increased pressure when the carapace of the animal was depressed manually. Clearing the catheter blockage was accomplished by withdrawing the needle and back-flushing the catheter. In general, leakage of hemolymph around the catheter was minimal, but experiments were terminated if leakage was noted.

Statistical Analysis

Baseline, peak, and gut hydrostatic pressure and peak force were calculated for each crab and averaged for soft-shell and hard-shell stages. All data were tested for normality using a Shapiro-Wilcoxon test. Pressures and peak forces passed the test for normality ($P > 0.05$) and were therefore compared using a paired Student's t-test. All statistical analyses were performed using JMP 5.1 (SAS Institute Inc.).

Results

Recordings from soft-shell crabs, less than 12 hours following exuviation, showed significant transient changes in pressure in the merus that are strongly correlated with changes in force (Figure 19). Similar force and pressure records were obtained for all soft-shell crabs measured (n = 14). The magnitude of hydrostatic pressure peaks correlate with the magnitude of force peaks measured ($r^2 = 0.82$, n = 10) (Figure 20).

Recordings from hard-shell crabs, 7 days following exuviation, show no significant changes in pressure corresponding to the large peaks of force (Figure 21). This was consistent among all crabs measured (n = 9).

The average pressure and maximum force change significantly as crabs transition from soft- to hard-shell stages (Figure 22). The average absolute pressure in the cheliped of soft-shell crabs was 4786 Pa (S.D. = 2019 Pa, n = 13), which was significantly greater than that of hard-shell crabs (2940 Pa, S.D. = 1551 Pa, n = 7) (Student's t-test, $P < 0.05$). The average peak force during cheliped flexure was an order of magnitude greater in hard-shell crabs (0.55 N, S.D. = 0.12 N, n = 7) than in soft-shell crabs (0.04 N, S.D. = 0.02 N, n = 13).

Recordings of gut pressure from newly molted crabs show a strong correlation between the pressure in the cheliped and gut during cheliped flexure (Fig. 23). Peaks of increased

pressure in the gut correspond with pressure peaks in the cheliped. Similar pressure records were obtained for all soft-shell crabs measured (n = 7). The average baseline pressures within the cheliped and the gut were not significantly different (cheliped: 3792 Pa, S.D. = 1029 Pa, n = 7; gut: 2737 Pa, S.D. = 1329 Pa, n = 7; Student's t-test, P = 0.12) (Figure 24), nor were the average maximum peak pressures within the cheliped and gut during cheliped flexion (cheliped: 808 Pa, S.D. = 563 Pa, n = 14; gut: 1088 Pa, S.D. = 510 Pa, n = 14; Student's t-test, P = 0.18).

Removing air from the gut causes a decrease in baseline pressure in both the gut and cheliped (Figure 25). The average baseline pressure in the gut decreased from 2113 Pa (S.D. = 1343 Pa, n = 8) to 828 Pa (S.D. = 434 Pa, n = 8) while that within the cheliped decreased from 2213 Pa (S.D. = 1318 Pa, n = 8) to 981 Pa (S.D. = 736 Pa, n = 8) (Figure 26). The pressures within the cheliped and gut are not significantly different before or after the withdrawal of air (Initial: P = 0.89, S.D. = 1330 Pa; Final: P = 0.64, S.D. = 604 Pa; Student's t-test, n = 7).

Figure 19

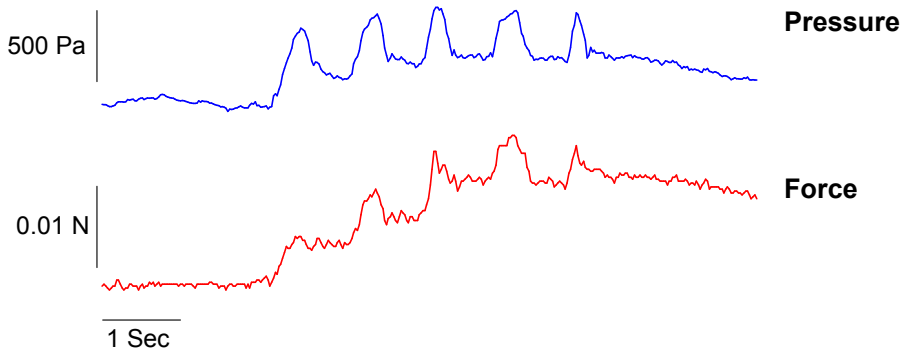

Figure 19. A representative recording of pressure and force from a soft-shell crab less than 12 hours after exuviation. The upper trace (Blue) is pressure and the lower trace (Red) is force. Peaks of increased pressure correlate with peaks of force. The traces represent approximately 9.5 seconds of recording.

Figure 20

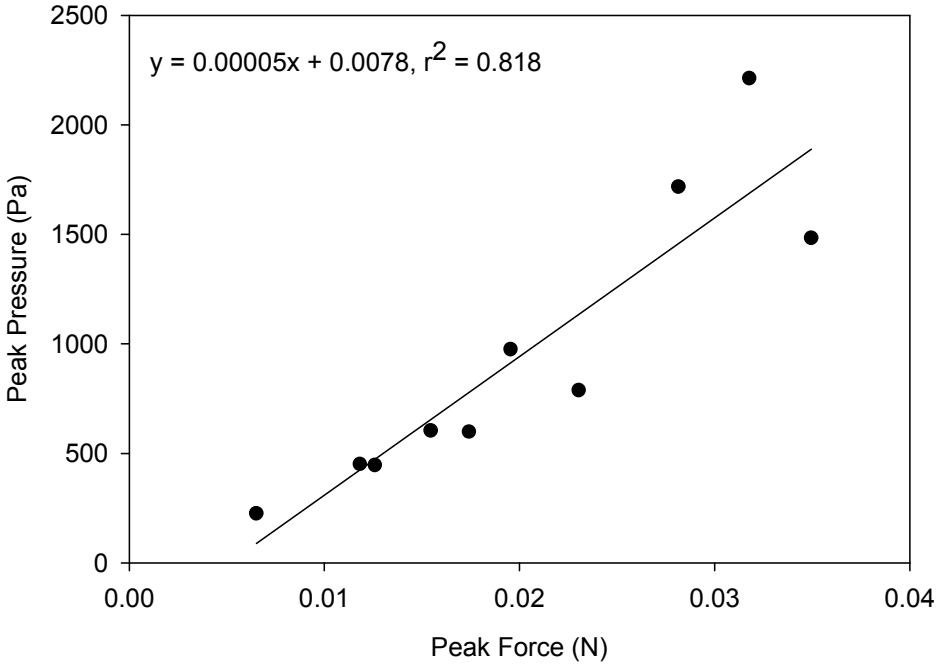

Figure 20. A plot of maximum pressure versus corresponding maximum force in soft-shell crabs. The data were fitted with a least squares regression. The equation for the regression and the r^2 value are listed on the upper left of the plot. The magnitude of pressure peaks correlates with the magnitude of force peaks. n = 10.

Figure 21

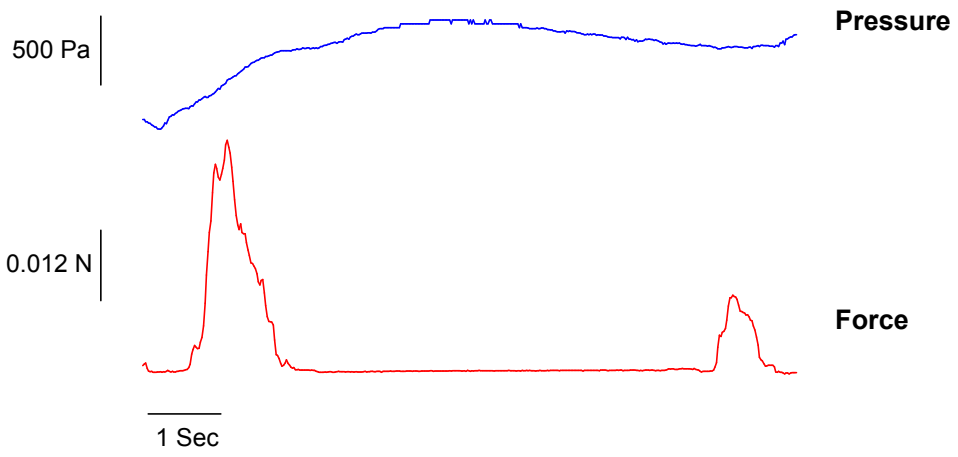

Figure 21. A representative recording of pressure and force from a hard-shell crab 7 days after exuviation. The upper trace (Blue) is pressure and the lower trace (Red) is force. Large forces are present, but there are no corresponding increases in pressure. The traces represent approximately 9.5 seconds of recording.

Figure 22

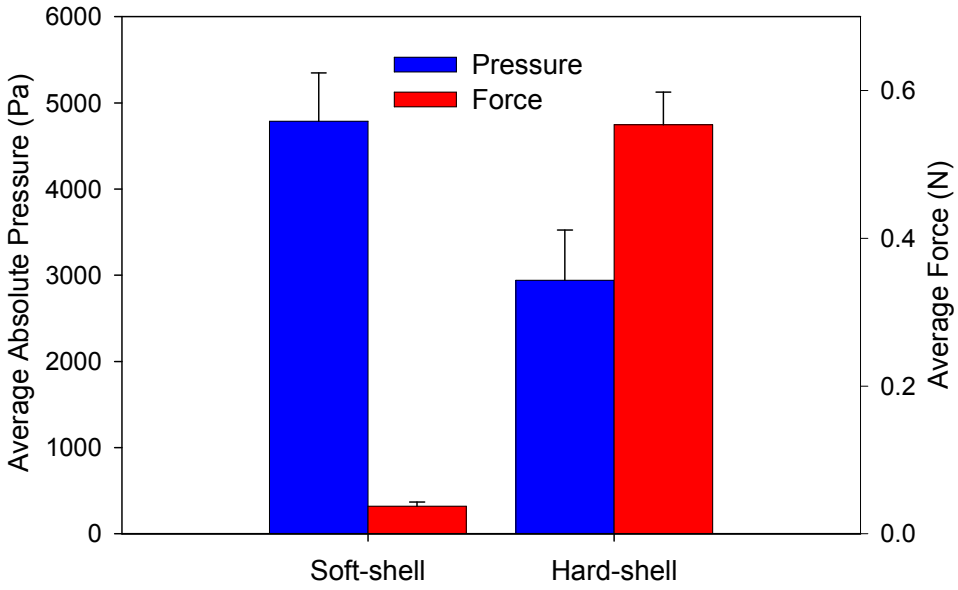

Figure 22. Average absolute pressure and force for soft-shell and hard-shell crabs. Absolute pressure is baseline plus peak. Pressure decreased significantly from soft-shell to hard-shell (Student's t-test, $P < 0.05$, $n = 13, 7$, respectively), while force increased significantly (Student's t-test, $P < 0.001$, $n = 13, 7$, respectively). Error bars = SEM.

Figure 23

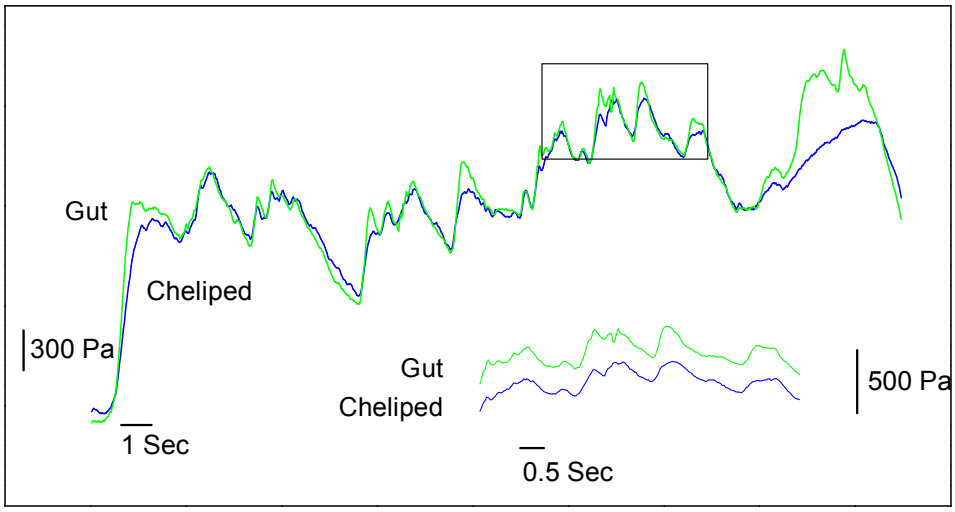

Figure 23. A representative recording of pressure in the gut and cheliped of a postmolt crab less than 12 hours after exuviation. The green trace is gut pressure and the blue trace is cheliped pressure. Baseline pressure and pressure peaks in the gut correlate with the baseline and pressure peaks in the cheliped. Inset is an expanded view of the larger trace, showing the correlation between individual peaks of pressure in the cheliped and gut during cheliped flexion. The large trace represents approximately 35 seconds of recording.

Figure 24

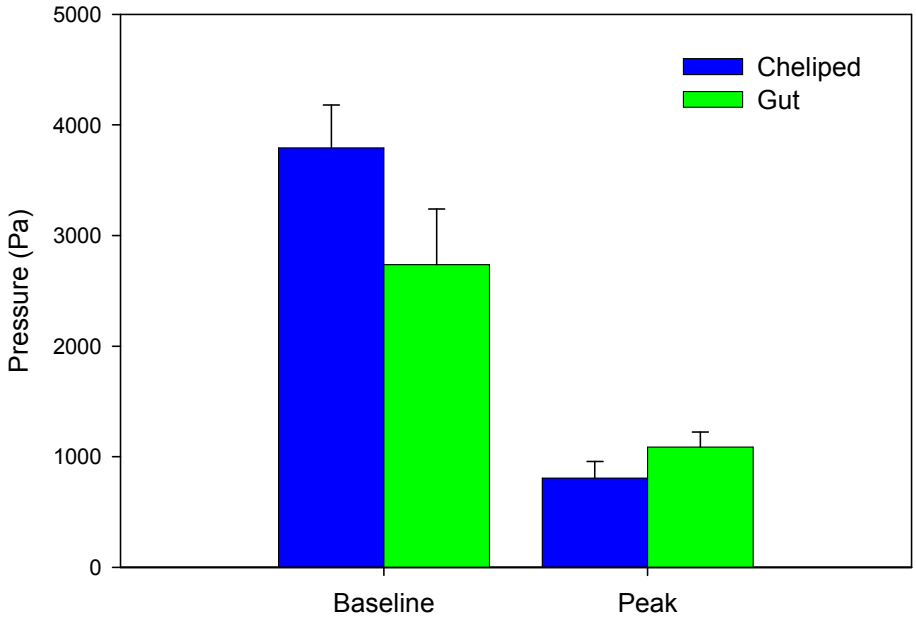

Figure 24. Average baseline and peak pressures in the gut and cheliped of a postmolt crab less than 12 hours after exuviation. The average baseline pressure in the gut was not significantly different from that in the cheliped (Student's t-test, $P > 0.05$, $n = 7$), nor was there a difference in the peak pressure (Student's t-test, $P > 0.05$, $n = 14$). Error bars = SEM.

Figure 25

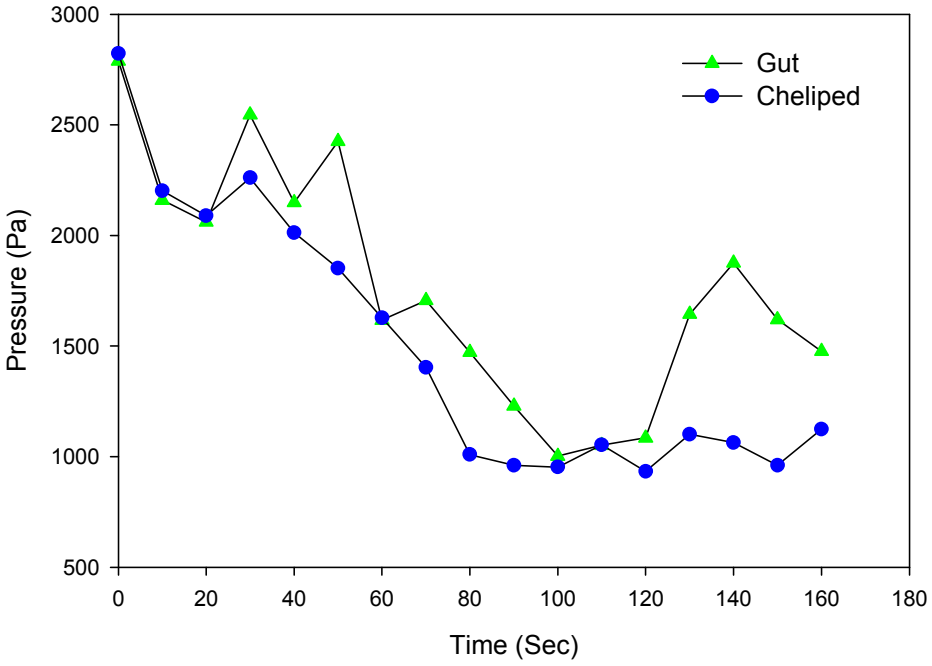

Figure 25. A representative recording of pressure in the gut and cheliped during air removal in a postmolt crab less than 12 hours after exuviation. Total duration of air removal was 160 seconds and pressures in the gut and cheliped were recorded every 10 seconds. Pressure in the cheliped decreases as gut pressure decreases during air removal.

Figure 26

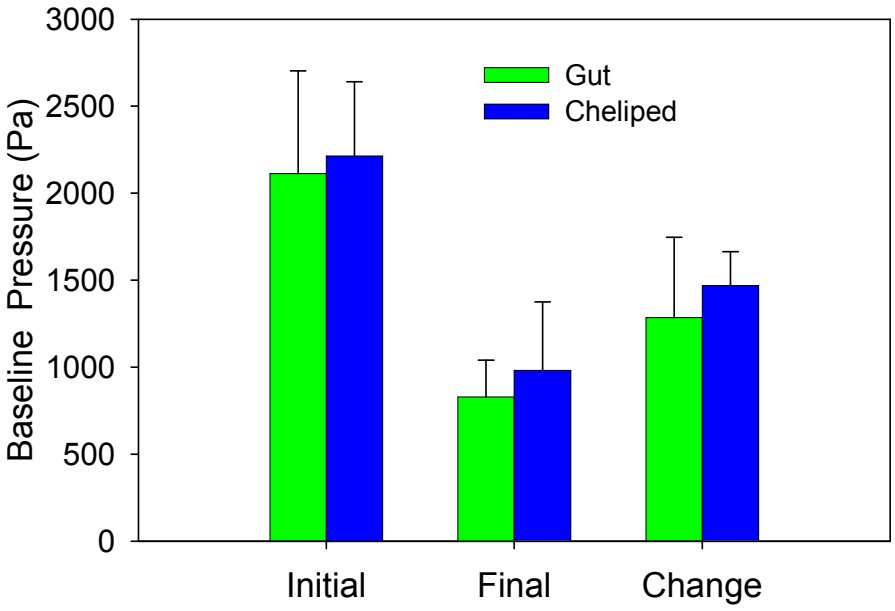

Figure 26. Average pressure in the cheliped and gut before and after gut air removal. Initial pressure measurements were taken before air was withdrawn and final pressure was taken after air removal was completed. Bars represent overall average for each treatment (initial and final). There was no significant difference between the initial baseline pressures in the cheliped and gut nor between the final baseline pressures in the cheliped and gut after air removal (Student's t-test, P = 0.89, P = 0.64, n = 7). Error bars = SEM.

Discussion

The strong correlation between internal hydrostatic pressure and the force of cheliped adduction in newly molted crabs is consistent with the use of hydrostatic skeletal support. When the adductor muscle in the merus contracts to bend the cheliped at the merus-carpus joint, it exerts a compressive force on the merus. This compressive force is resisted because the tension-resisting cuticle opposes the increase in diameter that would result from shortening of the constant volume segment (Clark and Cowey, 1958). Thus, muscle contraction is accompanied by an increase in hydrostatic pressure, the magnitude of which is proportional to the muscle force.

Following exuviation, the new cuticle begins to stiffen and the crab consumes the shed skeleton to obtain additional calcium for hardening the new cuticle (Skinner, 1985). After several days the cuticle hardens and is then sufficiently rigid to resist the compressive and bending forces of muscle contraction. Thus, it was predicted that at this point, no increase in hydrostatic pressure would be observed during muscle contraction. Indeed, recordings from crabs 7 days after exuviation are consistent with this prediction and show no increase in pressure as the carpus exerts force. These data imply that hydrostatic support is no longer used once crabs have hardened the new skeleton.

It therefore appears that blackback land crabs rely on hydrostatic skeletal support during molting, similar to the aquatic blue crabs (Chapter 1), despite the low water availability and significant gravitational forces associated with the terrestrial environment. Jones (1978) suggested that due to heavy loading, terrestrial hydrostatic animals must have higher internal pressures relative to aquatic animals in order to support themselves on land. Indeed, the highest baseline pressure recorded in the blackback land crab is significantly

greater than that of the aquatic blue crab, a pattern similar to that of some gastropods (Table 1). This suggests that a high internal pressure in terrestrial hydrostats is a significant biomechanical adaptation to life on land.

Another major terrestrial adaptation is the inflation of the gut with air. The pressures within the gut and cheliped of newly molted animals are tightly correlated, and when the air in the gut is removed, the pressures in the gut and cheliped decrease. This demonstrates that muscle contraction in the merus increases the hydrostatic pressure of the hemolymph throughout the body of the crab, as in a typical hydrostatic skeleton (Chapman, 1958, 1975; Clark, 1964; Kier and Smith, 1985). Since the gut wall is flexible, this results in an increase in pressure in the gut as well. These results are expected because the crab body is not compartmentalized and therefore local muscle contraction results in increased pressure throughout the body.

When air in the gut is withdrawn, body turgor is reduced significantly, and this has substantial impact on functioning of the animal. After air removal, the chelipeds and carapace become wrinkled and deformed (J. R. A. Taylor, personal observation). Animals that were able to move and lift their hefty claws before air removal, were no longer able to move them. This reduced functioning has also been observed in *Arenicola* when coelomic fluid was withdrawn (Chapman and Newell, 1947). The rate of burrowing in these worms was reduced in proportion to the volume of fluid removed, indicating the importance of having the appropriate fluid volume and body turgor for proper functioning.

These data support the hypothesis that the air within the gut increases body turgor and therefore plays a role in providing skeletal support in other regions of the postmolt crab body, such as the cheliped.

The increased hydrostatic pressure observed in molting arthropods has previously been considered important only for postmolt expansion (Shafer, 1923; Reynolds, 1980). But the pressurized body is essential for both shape and mobility. Greater hydrostatic pressure results in greater stiffness of the body, facilitating support and the transmission of muscular force (Wainwright, 1982). Both *G. lateralis* and *C. sapidus* are observed to be less mobile when they are less turgid (J. R. A. Taylor, personal observation).

The use of a gas as part of a hydrostatic support mechanism is unusual since the underlying principle of hydrostatic support involves a relatively incompressible, aqueous fluid. Gases are neither. Because gases are more compressible than liquids, the gut volume may be decreased significantly by an increase in hemolymph pressure due to muscle contraction. The inclusion of gas thus makes this system less effective in resisting the compressional forces of the musculature. Indeed, in postmolt *G. lateralis*, some folding of the cheliped is observed during forceful muscle contraction and this compression occurs without significant expansion in other areas of the body, as would be expected for animals with a conventional liquid-filled hydrostatic skeleton (Chapman, 1958, 1975; Clark, 1964; Kier and Smith, 1985).

Table 1

	Organism	Resting Pressure (Pa)	Habitat	Source
Crustacea	*Callinectes sapidus*	2,648	Aquatic	(Taylor, Chapter 2)
	Gecarcinus lateralis	6,700	Terrestrial	(Taylor, this Chapter)
Mollusca	*Viviparus viviparus*	805	Aquatic	(Jones, 1975)
	Lymnaea stagnalis	681	Aquatic	(Jones, 1975)
	Helix aspersa	1681	Terrestrial	(Jones, 1975)
	Helix pomatia	1725	Terrestrial	(Jones, 1975)

Table 1. Comparison of resting pressures in hydrostatic terrestrial and aquatic animals. Terrestrial species have higher hydrostatic pressures than aquatic species.

The presence of air in the gut of molting crabs has only been documented in *G. lateralis* (Bliss, 1956, 1968, 1979). Its prevalence among other terrestrial and semi-terrestrial crustaceans is unknown. However, air swallowing during molting has been described in several insect species (Shafer, 1923; Cottrell, 1962; Hughes, 1980; Reynolds, 1980; Miles and Booker, 1998). A pneumo-hydrostatic skeleton may thus be common in terrestrial arthropods. Its presence in the blackback land crab represents a potentially critical adaptation to life on land for the Crustacea.

Engineers use gases for support of objects such as tires, inflatable rafts, and domes. Arthropods are the only animals known to use a gas for skeletal support and locomotion. The use of a gas in terrestrial arthropods may be more than simply a physiological adaptation for low water availability; it may also be a biomechanical adaptation to the greater gravitational forces associated with life on land. Since air is less dense than water, a body inflated with air is easier to support compared with one filled with water. Pneumo-hydrostatic support may be one of the crucial adaptations made by arthropods during the evolutionary transition to land.

A compressible gas used in conjunction with an incompressible liquid to control movement is employed in many engineering applications. For example, the hydropneumatic car lifts used in service garages operate by applying air pressure to a reservoir of hydraulic fluid, which forces the hydraulic fluid to raise the lift and car. An analogous system has now been shown to function in a species of land crab. Since such a gas-liquid based skeleton does not fit the description of a classical or muscular hydrostat, it represents a new category of hydrostatic skeleton.

CHAPTER V

ONTOGENETIC SCALING OF RIGID AND HYDROSTATIC SKELETONS IN THE AQUATIC BLUE CRAB, *CALLINECTES SAPIDUS* (RATHBUN, 1896), AND THE TERRESTRIAL BLACKBACK LAND CRAB, *GECARCINUS LATERALIS* (FREMINVILLE, 1835)

Summary

Body size affects nearly every aspect of an animal's biology, including the form and function of the skeletal support system. For animals that alternate between two different skeletal support mechanisms, such as arthropods and other molting animals, the effects of scale are more complicated. Rigid and hydrostatic skeletons operate according to different principles, and each is likely to have individual constraints that will influence body form and function in distinct ways. This study was designed to explore the ontogenetic scaling of rigid and hydrostatic skeletons in an aquatic and a terrestrial crab species: the blue crab, *Callinectes sapidus*, and the terrestrial blackback land crab, *Gecarcinus lateralis*. Linear dimensions and cuticle thickness of the merus of the second walking leg were measured and related to body mass using reduced major axis regression. The external shape of the merus scaled isometrically for rigid and hydrostatic skeletons of both crab species. In blue crabs, the thickness of the cuticle in rigid animals scaled isometrically (slope: 0.39), while that of hydrostatic animals scaled allometrically (0.23). The same results were observed in blackback land crabs, except cuticle thickness decreases with body size for hydrostatic

animals (rigid: 0.39; hydrostatic: -0.17). It therefore appears that body size affects rigid and hydrostatic skeletons differently, and in ways that deviate from predictions. These results suggest that the intermittent use of hydrostatic skeletal support may be as significant of a biomechanical factor as the rigid exoskeleton in influencing growth to maximum body size in crabs.

Introduction

Body size, or scale, is fundamentally linked to the structure and function of animals. Scale impacts key physiological and biomechanical features of animals, such as metabolic rate, locomotion, and skeletal morphology (for review, see Alexander, 1971; Huxley, 1972; Pedley, 1977; McMahon and Bonner, 1983; Peters, 1983; Calder, 1984; Schmidt-Nielsen, 1984; Brown and West, 2000). Thompson (1961) recognized that animals must be able to support their own weight, and as they grow larger, both ontogenetically and evolutionarily, corresponding changes in shape must occur. This is because increases in size result in greater mass and locomotor forces that must be supported by the skeleton. As animals grow larger, body mass increases as the cube of the linear dimensions, while the strength of the skeleton increases as only the square of the linear dimensions (Swartz and Biewener, 1992). Therefore, in order to remain functional and support the increases in mass without failing, skeletons must be modified in either morphology or mechanical properties, or both (Schmidt-Nielsen, 1984); otherwise, animals must reduce their performance (Biewener, 2003).

Three models have been constructed to explain scale effects on the form and function of skeletal support systems: geometric similarity, elastic similarity, and constant stress similarity (see McMahon, 1973, 1975; McMahon and Bonner, 1983). Each model is based on

different scaling assumptions. Geometric similarity predicts that skeletal morphology does not change to accommodate increased loading during growth to larger size. Geometrically similar animals, therefore, have isometric growth, where the linear dimensions grow in proportion to each other. Thus, a given length, l, or diameter, d, is proportional to $W^{1/3}$, where W is body weight. To compensate for maintaining constant proportions throughout growth, geometrically similar animals must alter their skeletal mechanical properties, reduce their weight-specific loading, or reduce their performance to avoid failure (Schmidt-Nielsen, 1984; Biewener, 2003). Elastic similarity and constant stress similarity, on the other hand, require changes in skeletal morphology as animals grow larger. Elastic similarity assumes that the deflections due to forces remain constant as animals increase in size, while constant stress similarity assumes that the stresses experienced during locomotion remain constant (McMahon, 1984). Both of these models require allometric growth, where linear dimensions grow disproportionately to each other, but constant stress similarity predicts greater distortions in shape than elastic similarity (McMahon, 1984).

For most animals, growth is a continuous process, and the application of these scaling principles provides an understanding of how skeletal morphology and body size interrelate. In contrast, arthropods must repeatedly shed their external skeleton and secrete a new one to grow (see Herrick, 1895; Drach, 1939; Richards, 1951; Passano, 1960; Aiken, 1980; Skinner, 1985). During molting the animal remains soft for several days until the new skeleton hardens sufficiently to provide support for the animal. I showed recently that crabs support themselves during this time by switching to a hydrostatic (fluid-based) skeleton (Taylor and Kier, 2003, 2006). Generally, animals possess only one skeleton throughout their adult lives. The finding that crabs intermittently use a hydrostatic support mechanism in addition to their

characteristic rigid exoskeleton presents interesting new possibilities for how the arthropod body is affected by scale. Rigid and hydrostatic skeletons differ greatly in their structure and mechanism of support, and, thus, may follow different scaling principles, thereby influencing crab growth in different ways.

Rigid Skeletons

Rigid skeletal support systems, common in vertebrates and arthropods, are constructed as lever systems, which amplify either the force or displacement of muscle contraction. Rigid support systems are typically organized so that antagonistic muscles insert on stiff skeletal elements that move relative to each other at joints. The forces of muscle contraction are transmitted through the stiff elements, which, therefore, must resist the resulting compressive, bending, and torsional forces in order to avoid failure (Wainwright, 1982; Wainwright et al., 1982; Vogel, 2003). Flexural stiffness, *EI*, is a measure of the resistance to deformations resulting from bending forces (Wainwright at al., 1982). Resistance to bending can be increased by increasing the *EI* of the structure, where *E* is the elastic modulus, a material property, and *I* is the second moment of area, a morphological property. The second moment of area, *I*, defines how the material is distributed about the neutral axis, i.e. the mid-point where there is no stress. For rigid solid cylinders, *I* is proportional to the radius to the fourth power (Vogel, 2003). Assuming the same material properties, slight increases in the diameter result in significant increases in the resistance to bending. Rigid hollow cylinders, however, have a several-fold greater resistance to bending than solid cylinders of equal mass (Currey, 1967). The second moment of area, *I*, for rigid hollow cylinders is defined by

$$I = \frac{\pi}{4}(r_0{}^4 - r_i{}^4)$$

where r_0 is the outer radius and r_i is the inner radius. Thus, the thickness of the wall is an important component of flexural stiffness in rigid hollow cylinders.

For animals with exoskeletons, the larger the ratio of wall thickness to limb diameter, the greater the force that can be supported (Currey, 1967). Therefore in crabs, the diameter of the limb and thickness of the cuticle play a significant role in determining stiffness as body size increases. If the animal is to resist the increases in loading as it becomes larger, the cuticle thickness should increase disproportionately relative to other linear dimensions, i.e. allometrically. Indeed, Prange (1977) found that the thickness of the cuticle in cockroach legs scales allometrically.

Hydrostatic skeletons

Hydrostatic skeletons are arranged differently from rigid skeletons. Instead of using stiff elements to resist and transmit the forces of muscle contraction, hydrostatic animals use a constant volume of fluid and a tensile-resistant membrane. Hydrostatic skeletons are common in soft-bodied animals, such as earthworms, which are typically cylindrical in shape and have a flexible, muscular body wall surrounding a fluid-filled cavity (Chapman, 1958; Trueman 1975; Gutmann, 1981; Wainwright, 1970, 1982). The forces of muscle contraction are transmitted through the essentially incompressible fluid, resulting in an increase in hydrostatic pressure and body wall tension. The resting and dynamic pressures that occur during movement are the primary sources of stress on the skeleton, thus the body is primarily

loaded in tension. The tensile stress on the wall of a thin-walled cylinder is defined by Laplace's law, which is

$$\sigma_h = \frac{\Delta p r}{\Delta r}$$

where σ_h is the tensile stress in the hoop (circumferential) direction, Δp is the pressure differential, r is the radius, and Δr is the wall thickness. Again, the significance of wall thickness in supporting skeletal loading is obvious. To withstand the increased wall tension (Δpr) that occurs with increased body size, wall thickness must increase in proportion to the radius. For this reason, automobile tires have thicker walls than bicycle tires even though the pressures in bicycle tires are much higher (Vogel, 2003). It is therefore predicted that the thickness of the cuticle in hydrostatic crabs scales isometrically.

An earlier study on the scaling of hydrostatic skeletons in earthworms revealed that hydrostatic skeletons scale differently from rigid skeletons (Quillin, 1998). Specifically, earthworms grow isometrically, yet maintain elastic and constant stress similarity. For rigid skeletons, the former and latter two are mutually exclusive. This is possible in earthworms because the source of both static and dynamic stresses in hydrostatic skeletons is pressure, which scales independently of body mass (Quillin, 1998). It therefore appears that body size affects rigid and hydrostatic support systems in fundamentally different ways, but for both skeletons, cuticle thickness is a key factor. The cuticle thickness of rigid exoskeletons is predicted to scale allometrically while that of hydrostatic skeletons is predicted to scale isometrically. If crabs alternate between these two modes of skeletal support in order to grow, then both mechanisms of support play a key role in arthropod growth and provide different constraints for maximum body size.

Crustaceans

Most of our knowledge of the scaling of skeletons has been derived from studies on terrestrial mammals (e.g. Alexander et al., 1979; Prange et al., 1979). Much less attention has been focused on invertebrates, despite their abundance and considerable diversity in shape, skeleton, and behavior. There have been a few studies on the scaling of the exoskeleton in insects (Prange, 1977; Katz and Gosline, 1992, 1994) and many studies on crustaceans; however, they are mostly focused on the relative growth of sexually dimorphic characters such as the chelae and abdomen (for review, see Teissier, 1960; Hartnoll, 1974, 1978, 1982) and not skeletal support. This is surprising because crabs present a unique opportunity to evaluate directly the relationships between scale, body form, and the physical environment, given that they represent a large and diverse group of animals that inhabit a wide variety of aquatic and terrestrial environments.

Many of the scaling principles that are applicable to terrestrial animals are not applicable to aquatic animals. In the terrestrial environment, gravitational forces dominate; in the aquatic environment, gravity is negligible and hydrodynamic forces dominate. For terrestrial animals, increases in mass associated with growth to larger size result in increases in gravitational loading in addition to increases in locomotor forces. Thus, terrestrial animals may scale allometrically to accommodate these larger forces. For aquatic animals, increases in gravitational loading due to body mass are offset by buoyancy. Indeed, the buoyant support provided by water is both necessary and sufficient to support animals as large as a 100 ton blue whale (Schmidt-Nielsen, 1984).Thus, aquatic animals may not need to grow allometrically to support the increases in mass, unless the forces associated with movement and locomotion increase allometrically.

The maximum force exerted by a muscle is proportional to the cross-sectional area of the muscle. For geometrically similar animals, force scales isometrically, that is with body mass to the power of 2/3 (Hill, 1950; Alexander, 1985). But depending on the function of individual structures, the forces created during movement and locomotion may not scale isometrically. For instance, bite force scales with positive allometry in lizards (Herrel and O'Reilly, 2005) and burrowing forces scale with negative allometry in earthworms (Quillin, 2000). Despite the negative allometry in earthworm burrowing forces, muscle cross-sectional area scales with positive allometry, suggesting that forces should increase allometrically as well (Quillin, 2000). In American Lobsters and Snow Crabs, the surface area of the apodemes in the meropodites of the walking legs, which serve as a proxy for muscle cross-sectional area and thus force, scale with various levels of positive allometry (Mitchell and DeMont, 2003). This suggests that the muscle forces associated with locomotion in crustaceans increases allometrically. Thus, it is possible that aquatic animals, such as crabs, may still require allometric growth of the exoskeleton.

The evolution of the rigid skeleton contributed significantly to the successful exploitation of land by arthropods (Kennedy, 1927; Jones, 1978; Wainwright, 1982; Raven, 1985). But the rigid exoskeleton is temporarily replaced by a hydrostatic skeleton during each molt, and a hydrostatic skeleton is subject to additional difficulties in the terrestrial environment. Besides the increased risk of water loss, the soft cuticle of hydrostatic animals must support the additional water weight gained during molting, including the water temporarily stored in the gut or pericardial sacs that serves to inflate these structures and the body (Bliss, 1956, 1968; Bliss et al., 1966). This is more significant for animals in air than water, so a hydrostatic crab on land may have more difficulty maintaining body shape and

mobility as it grows larger and requires even greater volumes of water for molting. The weight of a hydrostatic skeleton may be as significant of a limiting factor for large crabs on land as the weight of a heavy exoskeleton. Indeed, terrestrial hydrostatic animals tend to be relatively small, e.g., annelids, insect larvae, gastropods, nematodes, platyhelminthes, and nemertines. The constraints imposed by both skeletal support systems may explain why the largest terrestrial crab reaches a 75 cm leg span (*Birgus latro*, Hartnoll, 1988) while the largest aquatic crab reaches a 3.8 m leg span (*Macrocheira kaempferi,* Cloudsley-Thompson, 1988).

In this study, we examine how rigid and hydrostatic skeletons scale in two species of crabs. We determine the scaling relationships of the merus of a walking leg in the aquatic blue crab, *Callinectes sapidus*, and the terrestrial blackback land crab, *Gecarcinus lateralis.* The goals of this study are to understand how the alternating use of two remarkably different skeletons influences crab growth and the effects that the physical environment has on the scaling relationships of these two support mechanisms.

Materials and Methods

Animals

Blue crabs

Blue crabs, *Callinectes sapidus,* were selected for this study because they are highly aquatic; the fifth pair of legs is specialized for swimming. In addition, they encompass a broad size range and are easy to obtain and to predict the timing of molting. Large male and female "peeler" crabs (within 2-3 days of molt) ranging from 63 to 90 mm carapace width

were obtained from O'Neals Sea Harvest, Wanchese, NC, USA. Small to medium crabs ranging from approximately 22 to 36 mm carapace width were collected from Masonboro Sound, Wilmington, NC. All crabs were transported to the University of North Carolina at Chapel Hill where they were maintained in individual seawater aquaria at a temperature of 19°C and a salinity of 15 - 20 ppt (Instant Ocean Artificial Seawater, Aquarium Systems Inc, Mentor, OH, USA). Peeler crabs were checked every 2 hours for the onset of exuviation, while small and medium crabs were checked multiple times daily.

Blackback land crabs

Blackback land crabs, *Gecarcinus lateralis,* were selected for this study because they are highly terrestrial; they only return to water to spawn and thus they molt without the buoyant support of water (Bliss and Mantel, 1968; Bliss et al., 1978; Hartnoll, 1988). Approximately 150 male and female land crabs, ranging from 36 to 58 mm carapace width, were collected from the Fajardo Reserve, Fajardo, Puerto Rico in June 2003 and August 2004 and shipped to the University of North Carolina at Chapel Hill. All crabs were maintained in environmental chambers at 27°C on a 12 h light:12 h dark cycle. Humidifiers were used to keep room humidity between 60 to 90%. Individual crabs were maintained in separate plastic containers with moistened sand and dishes of water. Containers were cleaned and crabs were fed carrots and lettuce twice a week and cat food once a week. The sand was changed regularly.

Since blackback land crabs do not have external changes in coloration indicative of molting as observed in blue crabs (Otwell, 1980), the proximity of molt was estimated by monitoring the growth of limb regenerates (Bliss and Mantel, 1968; Skinner and Graham,

1972). To induce limb regeneration, one leg, usually the smallest (pereopod 5), was removed from each crab by injecting a small amount of distilled water at the base of the leg.

Samples

The elapsed time following the molt was calculated from the time exuviation was complete, or if exuviation was not observed, was estimated based on the time of the previous check and the softness of the crab. Only healthy looking crabs with all appendages were used. The third walking leg (pereopod 4) was removed from crabs at either 1 hour (soft-shell stage) or 7 days (hard-shell stage) following molting, however, some measurements were made on hard blackback land crabs that were several months postmolt. The merus (the fourth, and often longest, segment) was separated and immediately fixed in either 10% Formalin and sea water for blue crabs, or 10% Formalin and deionized water for blackback land crabs. All fixed samples were removed from Formalin after several days and placed in Sorensens Buffered Phosphate 0.065 M for storage.

Measurements

Both male and female crabs were analyzed. Crab mass was measured using a balance prior to any limb removal. *C. sapidus* was patted dry and weighed in air. The length, width, and height of the merus of the right third walking leg (i.e. the largest leg, often leading in sideways locomotion) were measured using digital vernier calipers and a dissecting microscope with an ocular micrometer. Measurements of merus length were taken at the

midline on the anterior side of the segment, using landmarks for consistency. Merus height and width were both measured at the center of the segment.

For measurements of cuticle thickness, sections of the merus were cut transversely from the middle of the segment. Hard cuticle thickness was measured using a dissecting microscope with an ocular micrometer. Thirty measurements were taken around the circumference of the segment: anterior, posterior, dorsal, and ventral. Because the soft cuticle was too thin to measure accurately using calipers, cuticle thickness was measured using laser scanning confocal microscopy. The hypodermis was carefully removed from all samples using the edge of a razor blade under a microscope. The cuticle samples were then immersed in poly-L-lyseine for 20 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 surfaces. 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.

Analysis

Merus length, height, width, cross-sectional area, and cuticle thickness, and the ratio of cuticle thickness to merus diameter were each related to body mass in a log-transformed regression analysis, as per standard analyses of scaling (Swartz and Biewener, 1992). Slopes were calculated using Reduced Major Axis regression (RMA) software (Bohonak and van der Linde, 2004) which is a preferred method for calculating regressions because it accounts

for measurement error of data on both axes (Rayner, 1985; McArdle, 1988; Lovett and Felder, 1989). This software provides RMA slopes, intercepts, and 95% confidence intervals for the RMA slopes. A Student's t test was used to test the slopes of the regression lines against predicted slopes (β) of isometry, where H_0: $\beta = 0$ and H_A: $\beta \neq 0$, H_0: $\beta = 1/3$ and H_A: $\beta \neq 1/3$, and H_0: $\beta = 2/3$ and H_A: $\beta \neq 2/3$ (Zar, 1999). Regression lines were compared among stages and species using an ANCOVA, which tests for homogeneity of slopes (Zar, 1999). Finally, elevations of the regression lines were compared using a t-test (Zar, 1999).

Results

Merus shape and cuticle thickness

The merus of *C. sapidus* is elliptical in cross-section (Figure 27 inset). The hard cuticle varies in thickness around the perimeter of the merus (ANOVA, $P < 0.005$, $N = 12$) (Figure 27). The mean cuticle thickness of the anterior surface of the merus was significantly less than that of the dorsal and ventral surfaces (Tukey, $P < 0.05$), but not different from that of the posterior surface (Tukey, $P > 0.05$). Since the anterior and posterior surfaces of the merus comprise most of the elliptically shaped segment and were significantly different in thickness from the dorsal and ventral edges, measurements of cuticle thickness used in subsequent analyses were calculated from the average of the anterior and posterior surfaces.

The merus of *G. lateralis* is triangular in cross-section, with a thick, flat ventral surface (Figure 28 inset). The cross-sectional area was estimated as a triangle, which may be an underestimate of the actual cross-sectional area because the curvature of the segment was not accounted for. The hard cuticle varied in thickness around the perimeter of the merus (ANOVA, $P < 0.0001$, $N = 7$) (Figure 28). The mean anterior and posterior cuticle

thicknesses were significantly less than the dorsal and ventral cuticle thicknesses (Tukey, $P < 0.05$). For consistency, the anterior and posterior surfaces were averaged together and used for subsequent analyses of cuticle thickness.

Figure 27

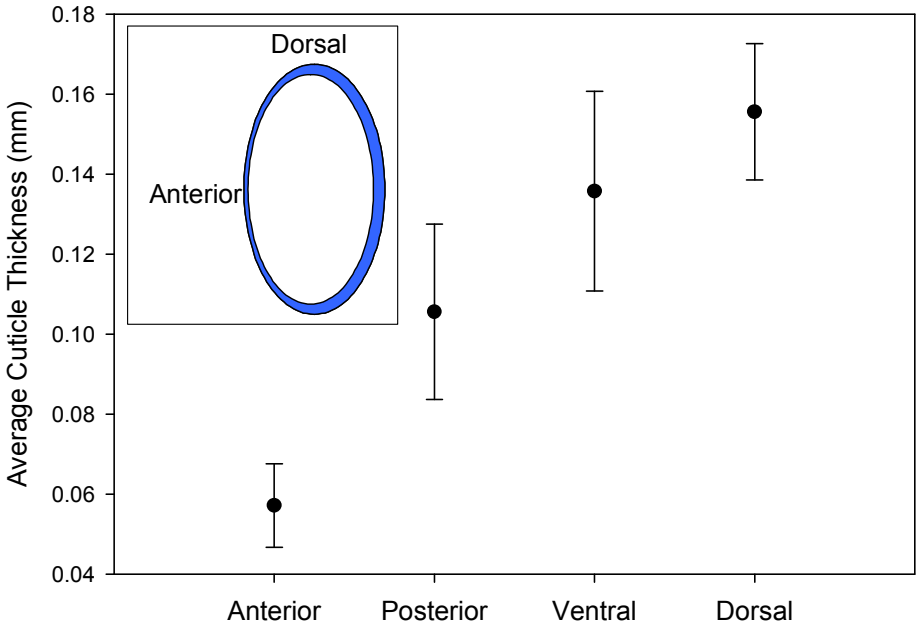

Figure 27. Average hard cuticle thickness of the merus of *C. sapidus.* Thirty total measurements were taken around the perimeter of a transverse section of the merus. Inset is a diagram of the cross-sectional shape of the merus. $N = 12$. Error bars represent standard error.

Figure 28

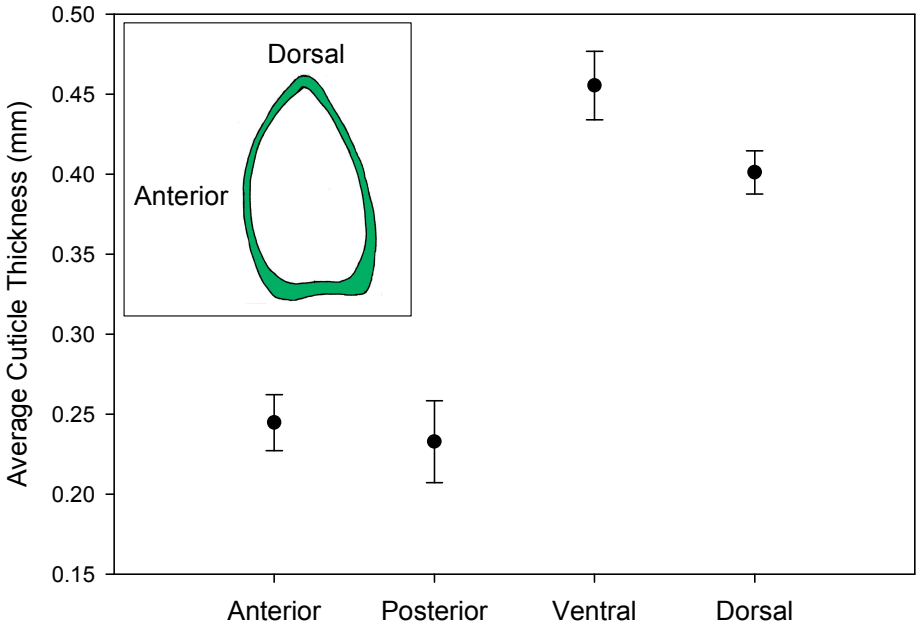

Figure 28. Average hard cuticle thickness of the merus of *G. lateralis.* Thirty total measurements were taken around the perimeter of a transverse section of the merus. Inset is a diagram of the cross-sectional shape of the merus. $N = 7$. Error bars represent standard error.

Scaling

Merus length

The length of the merus increases isometrically for hard-shell and soft-shell stages of both crab species (Figure 29). The slope for hard-shell *C. sapidus* was not significantly different from 1/3 ($b = 0.34$, t-test, $P = 0.36$, $t_{0.05(2),10} = 2.228$, $N = 12$), nor was the slope for soft-shell *C. sapidus* ($b = 0.31$, t-test, $P = 0.99$, $t_{0.05(2),8} = 2.306$, $N = 10$), hard-shell *G. lateralis* ($b = 0.21$, t-test, $P = 0.19$, $t_{0.05(2),5} = 2.571$, $N = 7$), and soft-shell *G. lateralis* ($b = 0.27$, t-test, $P = 0.34$, $t_{0.05(2),7} = 2.365$, $N = 9$). The 95% confidence intervals for *G. lateralis* were large compared to those of *C. sapidus* (Table 2). The regressions for hard-shell *G. lateralis* and hard-shell *C. sapidus* were the only ones with significantly different slopes (ANCOVA, $P < 0.05$). The elevation of the regression lines were significantly different for hard animals of each species (t-test, $P < 0.05$, $t_{0.05(2),16} = 2.120$) and soft animals of each species ($P < 0.05$, $t_{0.05(2),16} = 2.120$).

Merus Height

Merus height increases isometrically for hard-shell and soft-shell stages of both crab species (Figure 30). The slope for hard-shell *C. sapidus* was not significantly different from 1/3 ($b = 0.32$, t-test, $P > 0.05$, $t_{0.05(2),10} = 2.228$, $N = 12$), nor was the slope for soft-shell *C. sapidus* ($b = 0.27$, t-test, $P > 0.05$, $t_{0.05(2),8} = 2.306$, $N = 10$), hard-shell *G. lateralis* ($b = 0.20$, t-test, $P > 0.05$, $t_{0.05(2),5} = 2.571$, $N = 7$), and soft-shell *G. lateralis* ($b = 0.27$, t-test, $P > 0.05$, $t_{0.05(2),7} = 2.365$, $N = 9$). The 95% confidence intervals were large for both stages of *G. lateralis* (Table 2). None of the regressions had significantly different slopes (ANCOVA, P >

Table 2

Species	Stage	Measurement	Slope	SE	r^2	95% CI
C. sapidus	Hard	Merus Length	0.34	0.0079	0.995	0.320, 0.355
		Merus Width	0.32	0.012	0.987	0.290, 0.340
		Merus Height	0.32	0.0094	0.991	0.299, 0.341
		Cross-sectional Area	0.63	0.017	0.993	0.595, 0.673
		Cuticle Thickness	0.39	0.045	0.869	0.291, 0.491
		Thickness:Diameter	0.14	0.040	0.139	0.0477, 0.228
	Soft	Merus Length	0.31	0.0057	0.997	0.301, 0.327
		Merus Width	0.33	0.018	0.976	0.288, 0.370
		Merus Height	0.27	0.0094	0.991	0.252, 0.295
		Cross-sectional Area	0.60	0.013	0.997	0.569, 0.627
		Cuticle Thickness	0.23	0.029	0.873	0.164, 0.299
		Thickness:Diameter	-0.14	0.034	0.563	-0.223, -0.0668
G. lateralis	Hard	Merus Length	0.21	0.074	0.348	0.0148, 0.396
		Merus Width	0.25	0.090	0.331	0.0146, 0.475
		Merus Height	0.20	0.081	0.138	-0.0138, 0.405
		Cross-sectional Area	0.41	0.16	0.267	0.00648, 0.814
		Cuticle Thickness	0.39	0.090	0.733	0.159, 0.623
		Thickness:Diameter	0.40	0.16	0.232	-0.0032, 0.810
	Soft	Merus Length	0.27	0.038	0.864	0.182, 0.361
		Merus Width	0.22	0.071	0.259	0.0506, 0.388
		Merus Height	0.27	0.078	0.411	0.0845, 0.453
		Cross-sectional Area	0.43	0.12	0.443	0.142, 0.711
		Cuticle Thickness	-0.17	0.062	0.0158	-0.314, -0.0189
		Thickness:Diameter	-0.31	0.10	0.197	-0.551, -0.0609

Table 2. Summary of regression values for *C. sapidus* and *G. lateralis*. Regression slopes, standard error, r^2 values, and confidence intervals were calculated using reduced major axis regression.

Figure 29

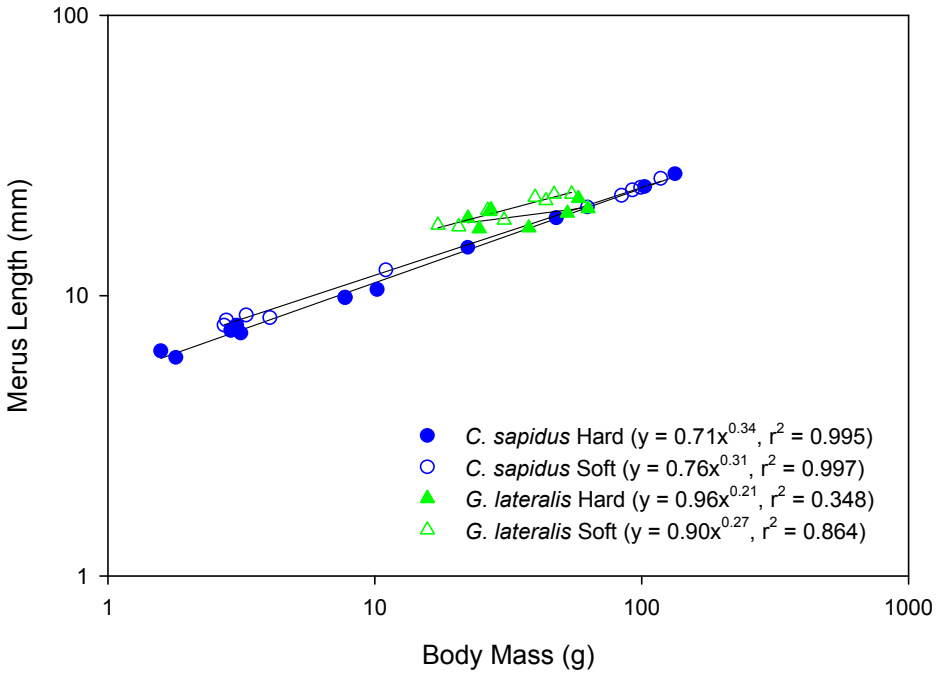

Figure 29. A logarithmic plot of merus length versus body mass for hard-shell and soft-shell *C. sapidus* and *G. lateralis*. Data were fitted using reduced major axis regression. The regression equations and r^2 values are given in the legend. Merus length is isometric for both stages and both species.

Figure 30

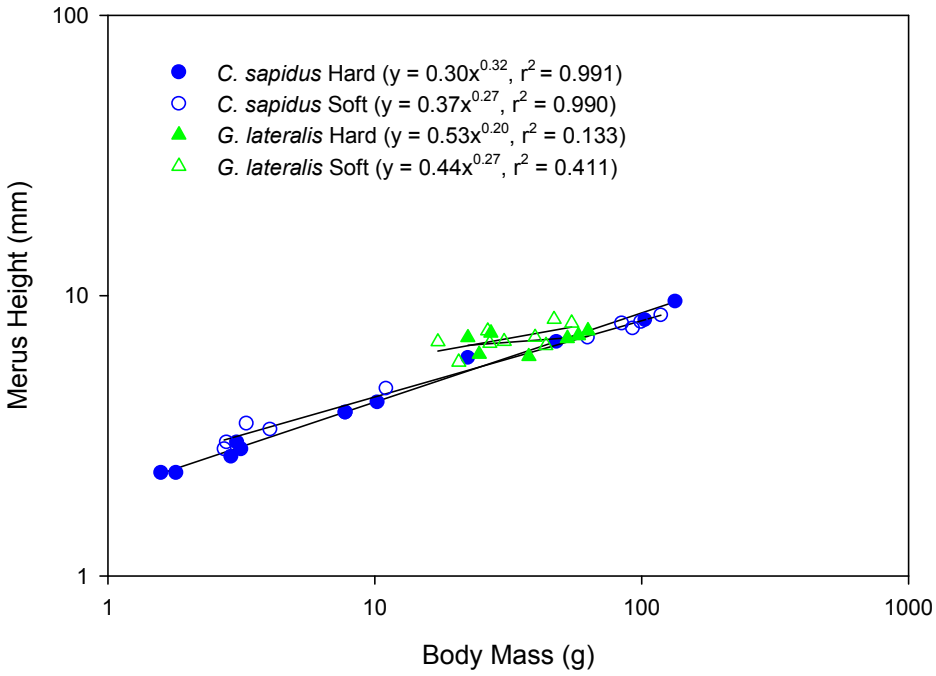

Figure 30. A logarithmic plot of merus height versus body mass for hard-shell and soft-shell *C. sapidus* and *G. lateralis*. Data were fitted using reduced major axis regression. The regression equations and r^2 values are given in the legend. Merus height is isometric for both stages and both species.

0.05) or elevations (t-test, $P > 0.05$), except for the different elevations of soft-shell *C. sapidus* and soft-shell *G. lateralis* ($P < 0.05$, $t_{0.05(2),16} = 2.120$).

Merus Width

The width of the merus increases isometrically for both stages of both crab species (Figure 31). The slope for hard-shell *C. sapidus* was not significantly different from 1/3 ($b = 0.32$, t-test, $P > 0.05$, $t_{0.05(2),10} = 2.228$, $N = 12$), nor was the slope for soft-shell *C. sapidus* ($b = 0.33$, t-test, $P > 0.05$, $t_{0.05(2),8} = 2.306$, $N = 10$), hard-shell *G. lateralis* ($b = 0.25$, t-test, $P > 0.05$, $t_{0.05(2),5} = 2.571$, $N = 7$), and soft-shell *G. lateralis* ($b = 0.22$, t-test, $P > 0.05$, $t_{0.05(2),7} = 2.365$, $N = 9$). The 95% confidence intervals for both stages of *G. lateralis* were large (Table 2). None of the regressions had significantly different slopes (ANCOVA, $P > 0.05$), except for the soft-shell stages of both species (ANCOVA, $P < 0.05$). The elevations were different for soft-shell stages of both crab species (t-test, $P < 0.0001$, $t_{0.05(2),16} = 2.120$) and hard-shell stages of both crab species ($P < 0.05$, $t_{0.05(2),16} = 2.120$).

Merus cross-sectional area

The cross-sectional area of the merus scales with isometry for both hard-shell and soft-shell *C. sapidus,* but the relationship was unclear for hard-shell and soft-shell *G. lateralis* (Figure 32). For *C. sapidus*, neither slope was statistically different from 2/3 (hard-shell: $b = 0.63$, t-test, $P > 0.05$, $t_{0.05(2),10} = 2.228$, $N = 12$; soft-shell: $b = 0.60$, t-test, $P > 0.05$, $t_{0.05(2),8} = 2.306$, $N = 10$) or from one another (ANCOVA, $P > 0.1$). For *G. lateralis,* the

regression for hard-shell animals had a slope of 0.41 (r^2= 0.267, N = 7) and the regression for soft-shell animals had a slope of 0.43 (r^2= 0.443, N = 9). ANCOVA indicated that neither of these slopes was different from 0.66 (P > 0.05) and the confidence intervals were large (Table 2). The regressions for hard-shell and soft-shell *G. lateralis* are not statistically different from one another (ANCOVA, P > 0.05). The slopes of both regression lines of *C. sapidus* are different from the slopes of the regressions of *G. lateralis* (ANCOVA, P < 0.005). Regression elevations only differed between soft-shell *C. sapidus* and soft-shell *G. lateralis* (t-test, P < 0.05, $t_{0.05(2),16}$ = 2.120).

Cuticle thickness

Cuticle thickness scales isometrically for hard-shell animals of both species (Figure 33). The slope of the regression line for cuticle thickness of hard-shell *C. sapidus* is 0.39 (r^2= 0.87, N = 12), which is not statistically different from 1/3 (t-test, $t_{0.05(2),10}$ = 2.228, P > 0.05). Soft-shell *C. sapidus* has a regression slope of 0.23 (r^2= 0.87, N = 10), which is different from 1/3 (t-test, $t_{0.05(2),8}$ = 2.306, P < 0.05). The regression slopes for hard-shell and soft-shell *C. sapidus* are significantly different from each other (ANCOVA, P<0.05).

The regression for cuticle thickness of hard-shell *G. lateralis* had a slope of 0.39 (r^2= 0.73, N = 7), which was not statistically different from 1/3 (t-test, $t_{0.05(2),5}$ = 2.571, P > 0.05). The regression for soft-shell animals had a slope of -0.17 (r^2= 0.02, N = 9), which was different from zero (t-test, $t_{0.05(2),7}$ = 2.365, P < 0.01) and 1/3 ($t_{0.05(2),7}$ = 2.365, P < 0.01). The regressions for hard-shell and soft-shell *G. lateralis* are significantly different from each other (ANCOVA, P < 0.01).

The regression slopes of hard-shell *C. sapidus* and hard-shell *G. lateralis* are not statistically different (ANCOVA, $P > 0.05$) but the regression slopes for soft-shell *C. sapidus* and *G. lateralis* are (ANCOVA, $P<0.05$). Additionally, the elevations of all regression lines were significantly different from one another (t-test, $P < 0.001$).

Ratio of cuticle thickness to limb diameter

The ratio of cuticle thickness to limb diameter (taken as merus width) scaled differently for hard-shell and soft-shell animals of both species (Figure 34). The regression for hard-shell *C. sapidus* had a slope of 0.14 ($r^2 = 0.139$, $N = 12$) while the regression for soft-shell animals had a slope of -0.15 ($r^2 = 0.563$, $N = 10$). Neither of these slopes were equal to zero (t-test, $t_{0.05(2),10} = 2.228$, $P < 0.005$; $t_{0.05(2),8} = 2.306$, $P < 0.001$) and they were significantly different from one another (ANCOVA, $P < 0.05$).

The regression for hard-shell *G. lateralis* had a slope of 0.40 ($r^2 = 0.232$, $N = 7$) while the regression for soft-shell animals had a slope of -0.31 ($r^2 = 0.197$, $N = 9$). A Student's t-test revealed that the regressions for both hard-shell and soft-shell animals are not different from either 1/3 ($t_{0.05(2),5} = 2.571$; $t_{0.05(2),7} = 2.365$, $P > 0.05$) or zero ($t_{0.05(2),5} = 2.571$; $t_{0.05(2),7} = 2.365$, $P > 0.05$) (Table 2).

Regression elevations were the same for soft-shell animals of both species (t-test, $t_{0.05(2),16} = 2.120$, $P > 0.05$), but were significantly different for all other regressions ($t_{0.05(2),19} = 2.093$, $t_{0.05(2),16} = 2.120$, $t_{0.05(2),12} = 2.179$, $P < 0.001$)

Figure 31

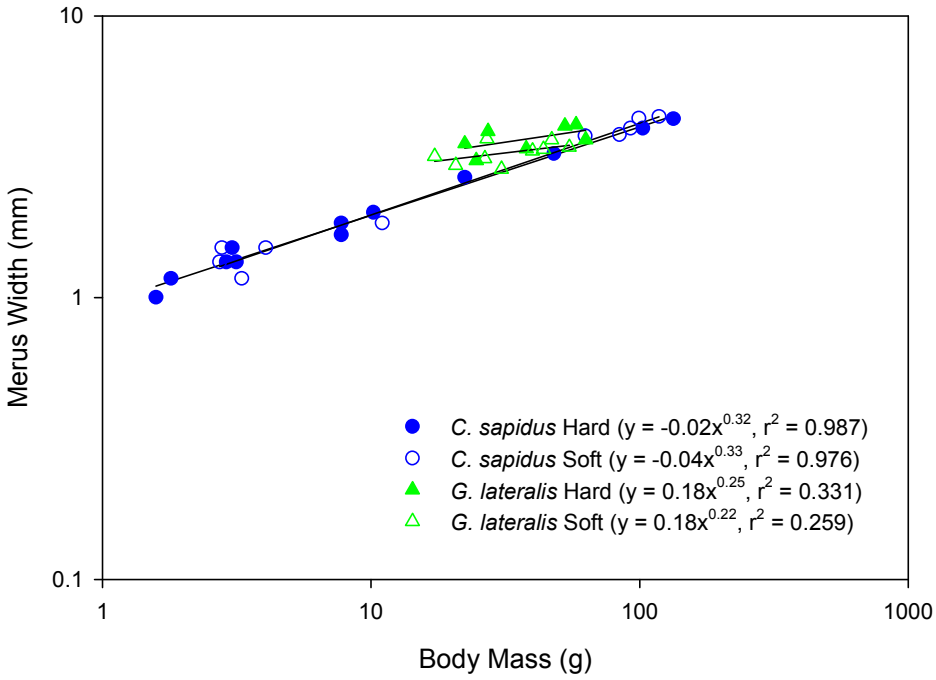

Figure 31. A logarithmic plot of merus width versus body mass for hard-shell and soft-shell *C. sapidus* and *G. lateralis*. Data were fitted using reduced major axis regression. The regression equations and r^2 values are given in the legend. Merus width is isometric for both stages and both species.

Figure 32

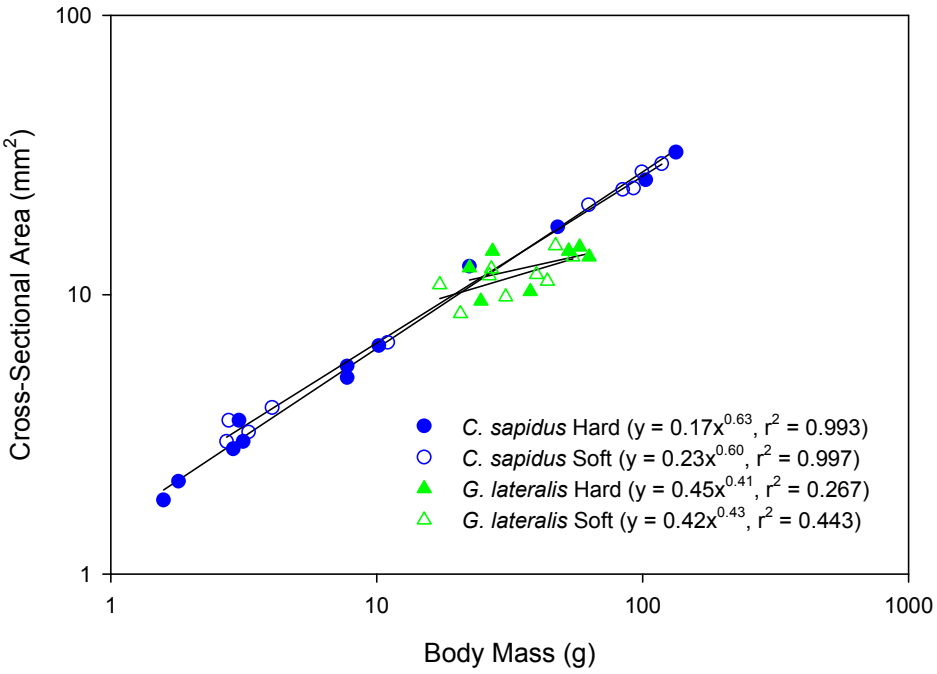

Figure 32. A logarithmic plot of merus cross-sectional area versus body mass for hard-shell and soft-shell *C. sapidus* and *G. lateralis*. Data were fitted using reduced major axis regression. The regression equations and r^2 values are given in the legend. Merus cross-sectional area is isometric for both stages and both species.

Figure 33

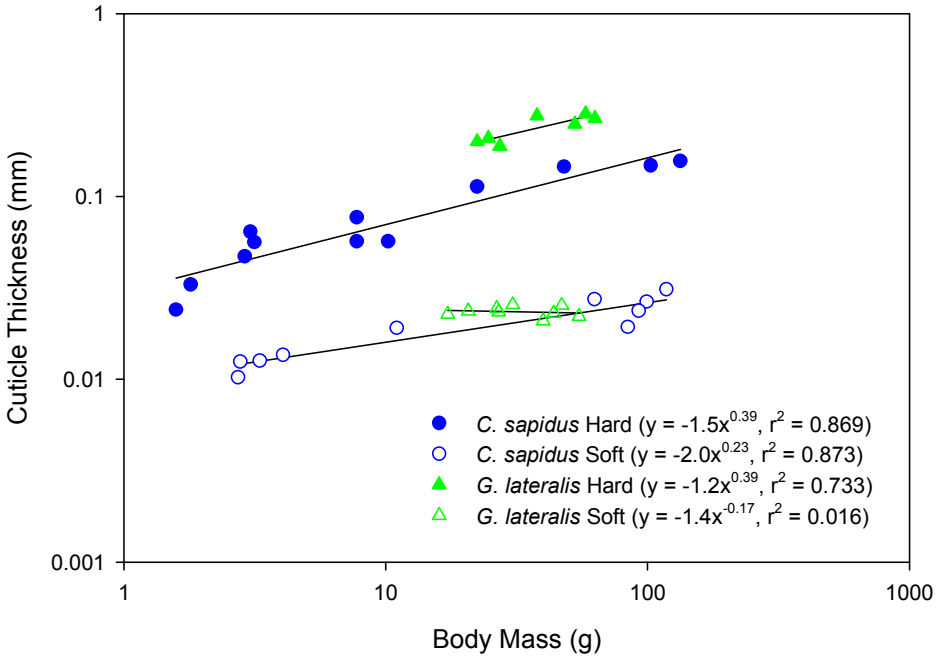

Figure 33. A logarithmic plot of merus cuticle thickness versus body mass for hard-shell and soft-shell *C. sapidus* and *G. lateralis*. Data were fitted using reduced major axis regression. The regression equations and r^2 values are given in the legend. Merus cuticle thickness is isometric for hard-shell animals of both species, but is negatively allometric for soft-shell animals of both species.

Figure 34

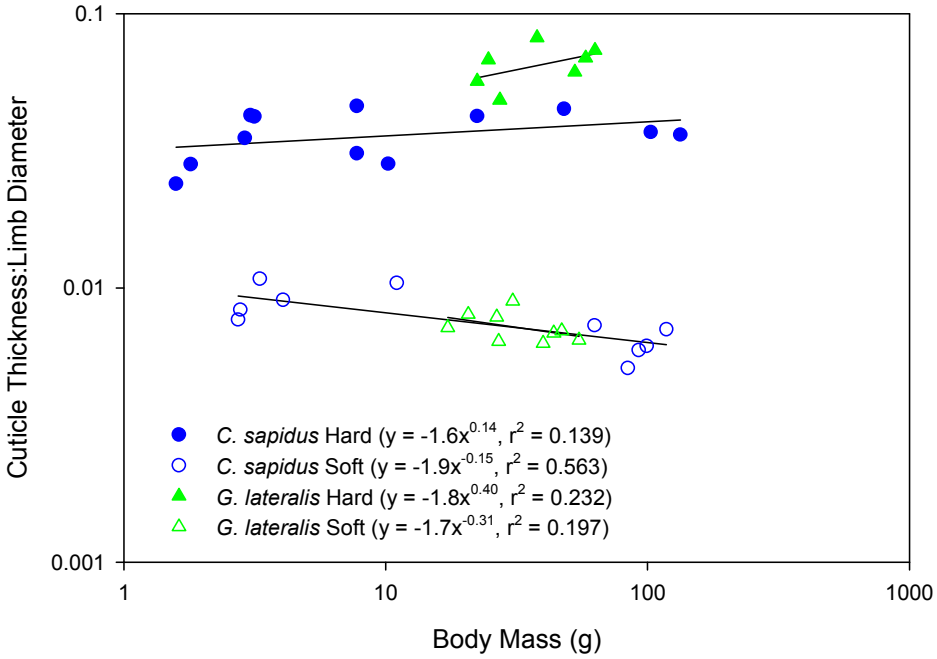

Figure 34. A logarithmic plot of the ratio of merus cuticle thickness to merus diameter versus body mass for hard-shell and soft-shell *C. sapidus* and *G. lateralis*. Data were fitted using reduced major axis regression. The regression equations and r^2 values are given in the legend. The ratio of cuticle thickness to diameter scales with positive allometry for hard-shell animals of both species and negative allometry for soft-shell animals of both species.

Discussion

Merus Shape

The external dimensions of the merus segment scale isometrically for both the rigid and hydrostatic skeletons of *C. sapidus* and *G. lateralis*. As crabs grow larger in size, the shape of the limb segment does not change, nor does it change during molting when animals switch from a rigid to a hydrostatic skeleton. This isometry in limb shape is consistent with previous studies on arthropod limb scaling, for example, that of the cockroach, *Periplaneta americana*, and the Wolf spider, *Lycosa lenta* (Prange, 1977). On the other hand, the metathoracic tibiae of the locust *Schistocerca gregaria* become more elongated during growth, although this reflects the specialization of this limb for jumping (Katz and Gosline, 1994). Though the molting process allows significant changes in shape to occur, including metamorphosis, the shape attained during expansion of the new cuticle immediately after molting provides the framework for the hardening of the new skeleton. If severe deformations are made in the soft cuticle and maintained during the hardening process, the rigid new skeleton will be formed with those deformations (personal observation). Thus, limb shape should not change once postmolt expansion is complete and there should be no difference in shape between the hydrostatic, newly molted animal, and the newly formed rigid exoskeleton. Since the external shape of the merus follows geometric similarity for both support mechanisms, the increases in static and dynamic loading associated with larger size must be supported by an alternative mechanism.

For hard-shell *G. lateralis,* each of the external dimensions scaled with slopes consistently lower than those predicted for isometry and those of *C. sapidus*, but statistically they did not differ from isometry. These lower slopes, in combination with the difficulties of

estimating cross-sectional area, resulted in the low regression slopes for the cross-sectional area (hard-shell: 0.41, soft-shell: 0.43). Furthermore, the regressions for merus length and height each had slopes slightly less than that of soft-shell animals. Taking the process of molting into consideration, it is unlikely that hard-shell crabs grow disproportionately smaller relative to soft-shell animals, as this would require shrinking before hardening. Rather, this suggests an inconsistency in measurements taken on rigid and soft animals. The non-significant differences in the various slopes for *G. lateralis* can be attributed to the fact that I was unable to obtain crabs smaller than 17 g or larger than 63 g, and therefore had only a one-fold size range, unlike the three-fold size range of *C. sapidus*. This narrow size range resulted in large confidence intervals, thereby making it difficult to resolve the true slope. The results for *G. lateralis*, therefore, should only be considered as a first approximation until measurements of additional crabs at each end of the mass scale can be performed.

Based on the elevations of the regression lines, the merus of *G. lateralis* is slightly greater in length, width, and height than the merus of *C. sapidus* of equivalent body mass, at least for the narrow size range measured. This may reflect differences in the function of the legs, since *C. sapidus* may rely more on the backfins for locomotion than the walking legs, while *G. lateralis* relies entirely on the walking legs for support and locomotion. Larger limbs could be advantageous for terrestrial crab locomotion because it increases stride length, which reduces the number of steps, and therefore cost, to move a certain distance (Taylor et al., 1970; Schmidt-Nielsen, 1984). Longer limbs are also more subject to buckling, but increasing the cross-sectional area of the limb will make it less susceptible to this type of failure. Of course, larger limbs also have greater skeletal mass that must be supported and

carried. This size difference may not be a mechanical adaptation but rather it may just reflect differences in the phylogenetic histories of the families Portunidae and Gecarcinidae.

Blue crabs, rigid skeleton

While the scaling of the external shape of the merus was isometric for both rigid and hydrostatic skeletons, the scaling of cuticle thickness differed. The thickness of the cuticle scaled with isometry in rigid *C. sapidus*, but with negative allometry in hydrostatic animals. Both of these outcomes differed from the predictions. For animals with rigid exoskeletons, I assumed that muscle-cross sectional area, and thus force, scales allometrically (Mitchell and DeMont, 2003). I therefore predicted that the cuticle thickness should scale allometrically, because support for the increases in locomotor forces associated with larger size requires disproportionate increases in wall thickness. However, these data show that hard cuticle thickness scales isometrically, similar to that of the Wolf spider, *Lycosa lenta* (Prange, 1977). This result could reflect a flaw in the assumption that force scales allometrically, which can be tested, or that *C. sapidus* uses an alternative mechanism to support the increased locomotor forces.

Animals with exoskeletons resist increases in bending loads by increasing *EI*. If *I*, the second moment of area, does not change, as is the case here where the cross-sectional shape remains constant, then stiffness can be increased by altering the material properties, *E*. So to compensate for isometric growth, the cuticle mechanical properties may change as a function of body mass. Such changes have been documented in the African Desert locust, where stiffness, *E*, scales allometrically (Katz and Gosline, 1994), but it is unknown if similar changes occur in the cuticle of blue crabs. Cuticle stiffness increases with body mass, i.e.

scales isometrically, for the limbs of other arthropods, such as the locust *Locusta migratoria migratorioides* (Hepburn and Joffe, 1974) and the millipede *Nyssodesmus python* (Borrell, 2004). For the cuticle mechanical properties to change as crabs grow larger, there must be underlying changes that occur in the composition or ultrastructure of the cuticle, but there is no known evidence of this. Clearly, arthropods have developed different mechanisms for dealing with scale effects on the exoskeleton and mechanical testing needs to be performed on the cuticle of crabs of a range of sizes to resolve this issue.

It is possible that the effects of scale are not manifested in the merus of the walking leg. Though blue crabs do crawl, they are specialized for swimming. The last pereopods are modified as swim paddles and are used in a sculling motion to propel the animal through the water. Crawling on the substratum likely occurs at low speeds, and the corresponding hydrodynamic forces on the animal are small (Vogel, 1994). If greater speeds are required, such as to escape predators, blue crabs tend to swim. Here the hydrodynamic forces will be more significant (Vogel, 1994). Thus, animals will experience greater locomotor stresses on the backfins during swimming than on the walking legs during crawling, which may also explain the observed isometry.

Furthermore, the ratio of cuticle thickness to limb diameter increased with body mass (slope: 0.14). This is significantly different from the expected value of zero for isometry, thereby contradicting the results that cuticle thickness scales with isometry. Additional crabs must be measured to reduce the variation and determine a more precise regression slope before these results can be considered conclusive.

Blue crabs, hydrostatic skeleton

The scaling of the hydrostatic skeleton of *C. sapidus* also did not follow the predictions. I predicted that cuticle thickness would scale isometrically, because, according to Laplace's Law, wall thickness should increase in proportion to the radius in order to support the resulting increases in wall tension. However, as body mass increases, cuticle thickness increases at a lower rate, scaling with negative allometry (slope: 0.23). The ratio of cuticle thickness to limb diameter, therefore, decreases with increased body mass, resulting in increasingly greater tension in the cuticle as the animal grows, assuming that hydrostatic pressure remains constant in growth.

During each molt, the cuticle is stretched to accommodate the larger body size, and wall tension is increased. If this increased tension is not supported by a proportionate increase in cuticle thickness, then either the internal hydrostatic pressure decreases as crabs get larger or the tensile properties of the cuticle change. For my predictions, I assumed that hydrostatic pressure does not change as a function of body size, based on Quillin's (1998) study on earthworms where she found no difference in pressure over the one order of magnitude mass range she measured. Internal pressure was also independent of body size in the sipunculid *Phascolosoma gouldi* (Zuckerkandl, 1950). It is possible that hydrostatic pressure differs for small and large crabs, but this is unknown. Indeed, small animals can withstand higher internal pressures than large animals (Vogel, 2003), as exemplified in the high pressures recorded for the nematode *Ascaris* (Harris and Crofton, 1957). Decreases in pressure with size could explain why wall thickness does not grow in proportion to radius, but further measurements are required of the resting hydrostatic pressures in newly molted crabs of a range of sizes to resolve this issue.

If hydrostatic pressure does not change as a function of size, the mechanical properties of the soft cuticle may change to withstand greater wall tension. Specifically, the tensile strength of the cuticle must increase with increase in body mass, i.e. scale isometrically, to balance the relatively thinner cuticle or larger animals. Though several studies have examined the mechanical properties of crustacean cuticle (Hepburn et al., 1975; Joffe et al., 1975a,b; 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 of soft cuticle (Dendinger and Alterman, 1983; Dutil et al., 2000; Taylor et al., in preparation) and I know of no studies of the mechanical properties of soft cuticle as a function of body size. It is therefore unknown if the tensile properties of soft cuticle change with body mass. It is unclear how the tensile properties would change, unless the cuticle composition also changes during growth. Measurements of the mechanical properties of both soft and hard cuticle of crabs of a range of sizes would, therefore, yield valuable information.

If neither the internal pressure nor the cuticle mechanical properties change as a function of body size, then the effects of scale may be manifested in reduced locomotor performance. It was a long standing assumption that newly molted crabs were incapable of movement (Passano, 1960; Bliss, 1962; Ryer et al., 1997). But impressive displays of movement and locomotion have been described for a variety of newly molted crabs, lobsters, and stomatopods (Olmsted and Baumberger, 1923; Travis, 1954; Lipcius and Herrnkind, 1982; Steger and Caldwell, 1983; Adams and Caldwell, 1990; Cromarty et al., 1991; Phlippen et al., 2000). Though locomotion is maintained throughout the molting process, there have been several observations of reduced locomotor performance in newly molted

animals. For instance, jumping performance is significantly reduced during molting in grasshoppers (Gabriel, 1985a; Queathem, 1991), and the lobster, *Homarus americanus*, readily performs an escape response while soft, but the tail flips have reduced force, velocity, and acceleration compared to the hard-shell stage (Cromarty et al., 1991). Large blue crabs are noticeably slower immediately following molting than small animals, which can swim without any noticeable difference in speed (personal observation). The observed scaling relationship of the soft-shell crab could, therefore, limit swimming speed and acceleration.

Additionally, it is possible that there was bias in the measurement of cuticle thickness. Although all animals were measured while the cuticle was still entirely soft, it was more difficult to record the precise time of molt for small blue crabs. And since the molting process is more rapid in smaller individuals, it is possible that measurements of small crabs were made after some or all of the endocuticle had been deposited. This would bias the small crabs toward thicker cuticles, making the regression slope shallower.

Blackback land crabs, rigid skeleton

Unlike their aquatic counterparts, *G. lateralis* must deal with significant gravitational forces in addition to the forces produced during locomotion. Thus, increases in mass during growth have a larger impact on the form of the skeletal support system for terrestrial animals. I predicted that the cuticle thickness of *G. lateralis* would scale allometrically, increasing at a slightly greater rate than body mass. However, the thickness of the cuticle in hard-shell crabs scales isometrically, like that of *C. sapidus*. Interestingly, the slopes of the regressions for hard cuticle of both crab species were identical (slope: 0.39). This is a surprising result that suggests that scaling of cuticle thickness is consistent among crabs and is therefore a

conserved aspect of the exoskeleton that is unaffected by the difference in forces imposed by terrestrial and aquatic environments.

The slope of 0.39 is slightly greater than the 0.33 predicted for isometry, though they are not statistically different. Furthermore, the ratio of cuticle thickness to limb diameter increased with body mass (slope: 0.40). The slope is high compared to the expected slope of zero, if both dimensions scale with isometry. But this slope was actually not statistically different from zero, indicating how high the variation was. It is, therefore, difficult to say if this allometric scaling is real. If cuticle thickness does scale with isometry, then as the loading on the skeleton increases during growth, some other parameter must change, such as the cuticle mechanical properties.

In general, the cuticle of rigid blackback land crabs is thicker than that of blue crabs of equivalent size. The difference in cuticle thickness may reflect the differences in loading between the two environments or other important physiological factors, such as water loss. If the cuticle were to increase in thickness at a greater rate than the increase in mass, i.e. allometrically, then crabs would have disproportionately more skeletal mass to carry around. A heavy skeleton would affect the buoyancy of aquatic crabs, but it would be even more costly for terrestrial crabs. Selection against a heavy skeleton may, therefore, preclude allometry in cuticle thickness.

Blackback land crabs, hydrostatic skeleton

In soft-shell land crabs, cuticle thickness was predicted to scale isometrically. Instead, it decreased with increased body mass. This is counter to Laplace's law and to what is known about the structure of the cuticle. This result suggests that the epi- and exo-cuticle layers,

which are present immediately following molting, become thinner as the animal grows, which is unlikely. Rather, it is more likely that these cuticle layers either remain constant in thickness or increase slightly. If ordinary least squares regression is used for this analysis, which ignores error in the x-axis data, then soft cuticle thickness is independent of body mass ($b = 0.024$, $r^2 = 0.021$). Clearly, narrow size range is a problem for determining the slope of this relationship. The only conclusion that can be drawn from this small data set is that soft cuticle does not scale isometrically as predicted, as also observed in blue crabs (slope: 0.23).

Since cuticle thickness does not scale isometrically for hydrostatic land crabs, the problem of supporting large body size is even more severe. First, greater pressures are required to support terrestrial hydrostats (Jones, 1978), and indeed, the average resting pressure in hydrostatic *G. lateralis* is greater than that of *C. sapidus* (Taylor and Kier, 2003, 2006; Chapters 1 and 3). Pressure is not likely to decrease in larger animals and reduce wall tension. Rather, larger animals will require larger pressures to maintain their body shape under the increased effects of gravity. Quillin (1998) excluded gravity from her scaling analysis of earthworms since it was insignificant for their low vertical height. For land crabs, however, body weight presents a significant source of loading; not only are the animals much larger, but the body is held above the substrate by the legs. Unless the tensile properties of the cuticle change, then locomotion of hydrostatic land crabs will be jeopardized.

Rigid *G. lateralis* can run very quickly and easily across a variety of substrates. Hydrostatic animals, on the other hand, are unable to hold their bodies off the substrate and move very slowly; they use their large chelipeds to drag their bodies across the surface (personal observation). They are capable of lifting their large heavy claws off the substrate, but their legs are not strong enough to support the body. Small hydrostatic crabs, those less

than approximately 25 to 30 g, not only support their bodies off the substrate with their legs but can crawl relatively quickly (personal observation). Though hydrostatic animals can maintain their body shape with increases in body size, there is a severe cost in terms of locomotion. This limitation in locomotor performance was also observed in the blue crabs, but is more extreme in the land crabs. This is different from earthworms, in which burrowing and crawling are kinematically similar (Quillin, 1999, 2000). In general, locomotion in terrestrial hydrostatic animals, for instance caterpillars and dipteran larvae, is many times more costly than that of arthropods of similar size with rigid exoskeletons (Casey, 1991; Berrigan and Lighton, 1993). This high cost of locomotion probably increases with body mass and may be a consequence of the scaling of the hydrostatic skeleton.

As mentioned previously, *G. lateralis* does not molt in water. Instead, these crabs molt in debris, near logs, or in burrows that do not reach the water table (Bliss and Mantel, 1968; Bliss et al., 1978). Instead of inflating with only water during molting, they swallow air to inflate the gut (Bliss, 1968, 1979), a strategy common in insects (Shafer, 1923; Cottrell, 1962; Hughes, 1980; Reynolds, 1980; Miles and Booker, 1998). Because air is significantly less dense than water, inflating the body by temporarily filling the gut with air instead of water reduces the weight gain during molting considerably. This may allow land crabs to expand to a larger body size without the proportionate increase in mass, thereby putting less stress on the hydrostatic skeleton. Though swallowing air is considered to be an adaptation to low water availability, it is possible that it is also a mechanical adaptation to life on land. Indeed, there is a trend for terrestrial animals to minimize the amount of water supported against gravity (Wainwright, 1970). There are many terrestrial and semi-terrestrial crab

species with diverse ecologies (Hartnoll, 1988), and a survey of molting might elucidate this and yield other possible mechanical adaptations related to scale and skeletal support.

Conclusions

In this study, I analyzed the scaling of a support structure in crabs, i.e. the merus of a walking leg, and observed that the external shape does not change with size. Cuticle thickness grows disproportionately for hydrostatic skeletons (slope: 0.23 and -0.17), but not for rigid skeletons (slope: 0.39). This was consistent for the aquatic and terrestrial crabs measured and indicates that the two skeletal support systems scale differently. Furthermore, these results suggest that the physical environment has a greater effect on hydrostatic skeletons than rigid exoskeletons.

The finding that rigid skeletons scale with the same slope in both *C. sapidus* and *G. lateralis* is surprising. This may be partially explained by the differences observed in cross-sectional shape of the merus. Interestingly, in *C. sapidus* the merus cross-sectional shape is elliptical, with thicker dorsal and ventral edges. This shape adds increased resistance to bending by affecting the second moment of area along the dorsal-ventral axis of the limb segment (Wainwright et al, 1982), suggesting that this is a major source of loading on this particular segment. In the land crab, the cross-sectional shape of the merus is triangular with a thick, flat ventral surface. This shape provides even greater resistance to bending along the dorsal-ventral axis, and the flat ventral surface serves as a thick, tensile resistant sheet. This cross-sectional shape, with a flat ventral surface, is analogous to the I beam frequently used in engineering. I beams are particularly resistant to bending along a particular axis because the I-shaped cross section places most of the material away from the neutral axis and

concentrates it in regions where the tensile and compressive stresses are maximal. The merus of the land crab likely incurs significant bending forces. Additionally, the cuticle of *G. lateralis* is significantly thicker than that of *C. sapidus*, imparting even greater stiffness.

Cross-sectional shape is not as significant for the functioning of hydrostatic skeletons as it is for rigid skeletons. Most hydrostatic animals are cylindrical in shape, because this is the most economical shape for containing fluids under pressure (Jones, 1978). Hydrostatic skeletons with high internal pressures generally have round cross-sections. Changes in cross-sectional shape only occur when the animal is not maximally inflated as observed in many extensible worm bodies (Clark and Cowey, 1958; Jones, 1978). The merus cross-sectional shape did not change during the shift between hydrostatic and rigid skeletons for either species. For the hydrostatic merus to be elliptical or triangular in cross-section, the internal pressure is probably not maximal. Maintaining this non-circular cross-section must be accomplished by localized differences in the structure and mechanical properties of the cuticle, perhaps, for example, fiber orientation. It would therefore be interesting to measure the properties of the cuticle at the point of smaller radius of curvature.

Hydrostatic skeletons scaled with negative allometry in *C. sapidus* and *G. lateralis.* Both species experience significant decreases in locomotor performance with size, with scale effects being more dramatic in the land crabs.

It has been suggested that small animals should scale isometrically because the relatively small loads are not likely to cause failure of the skeleton or require changes in shape as in large animals (Bertram and Biewener, 1990). Scale effects, however, have been demonstrated in insects, such as the allometric thickening of the cuticle in *Schistocerca*

gregaria metathoracic tibiae (Gabriel, 1985b). This suggests that scale effects are important for animals as small as insects.

I only determined if crabs scaled isometrically or not, and did not evaluate scaling in the context of other scaling hypotheses, i.e. constant stress similarity and elastic similarity. Both of these models have specific predictions for the relationship of both lengths and diameters to body mass, but they were derived from studies on endoskeletons. Since exoskeletons and hydrostatic skeletons differ greatly from the endoskeletons, it is possible that these skeletal support systems may not follow the predictions of either of these models, as observed in an analysis of the morphology of the locust tibia (Katz and Gosline, 1992). It is also possible that parameters other than cuticle thickness may be more important in the scaling of exoskeletons, such as cuticle mechanical properties, muscle tension, and pressure. These should be measured in crabs of a range of sizes to determine the effects of scale more conclusively.

Though *C. sapidus* and *G. lateralis* are not closely related species (Families Portunidae and Geocarcinidae), they are both brachyurans that have remarkably different ecologies. Blue crabs are entirely aquatic and adapted for swimming while blackback land crabs are entirely terrestrial, only returning to water to spawn. They are thus good species to use for an initial examination of arthropod scaling in aquatic and terrestrial environments. Expanding this analysis to include more species, within a phylogenetic framework, would provide a better understanding of the evolution of scaling and mechanical adaptations in arthropods.

There are many examples of animals that possess two types of skeletons simultaneously. For instance, bivalve mollusks have a rigid shell for protection and a

hydrostatic foot for locomotion (Trueman, 1975) and elephants have a rigid endoskeleton for support and movement while the trunk operates with a muscular-hydrostatic mechanism (Smith and Kier, 1989). In these animals, only one skeleton serves the functional roles of support and locomotion. Crabs, and likely other molting animals, depend on two fundamentally different skeletal support systems throughout their adult life. This presents additional complications for understanding how body size relates to skeletal morphology. Rigid and hydrostatic skeletons function by different mechanisms and are affected by scale in different ways. Thus, the constraints of both skeletons will each likely influence crab growth. Which mechanism poses more significant constraints on body size is unclear. The mechanical limits to limb strength are thought to determine the upper limit to arthropod body size (Currey, 1967), but the size limitations of hydrostatic skeletons are unknown (Jones, 1978). In order to grow to a larger size, crabs must first successfully undergo ecdysis. If the hydrostatic animal cannot support its own weight and shape, severe deformations may occur, which will compromise the succeeding hardened skeleton. Thus, the hydrostatic stage may present the more limiting stage, as suggested by Kennedy (1927) for why insects are limited to small size. Whether or not this is the case, the hydrostatic skeleton used by crabs during molting should be recognized as a biomechanical characteristic of considerable importance in the growth to maximum size of crabs.

CHAPTER VI

CONCLUSIONS AND FUTURE DIRECTIONS

Several important conclusions can be drawn from this dissertation research. The first is that internal hydrostatic pressure varies in proportion to the force of muscle contraction in newly molted blue crabs, but not in animals with a rigid exoskeleton. This supports the hypothesis that crabs switch to a hydrostatic skeleton during molting. Furthermore, increases in pressure occur throughout the body during localized muscle contraction, indicating that the crab body is not compartmentalized. This suggests that the crab body, despite its arthropod characteristics, functions like that of a typical hydrostatic animal.

Second, the cuticle of blue crabs undergoes significant changes in mechanical properties throughout the molt cycle. The flexural stiffness is remarkably low in newly molted animals, but increases four orders of magnitude as the new skeleton hardens. Additionally, the Young's modulus in tension increases as the cuticle changes from soft to stiff, but the tensile strength does not change. These properties suggest that the cuticle of newly molted crabs cannot support the bending forces of muscle contraction, but can support the tensile forces of the hydrostatic skeleton. Therefore, the changes in cuticle mechanical properties facilitate the use of both rigid and hydrostatic skeletons.

Third, the internal hydrostatic pressure in the claw and gut and the force of muscle contraction are all tightly correlated in newly molted blackback land crabs. When air in the gut is removed, pressures throughout the body decrease, indicating that the air in the gut is

important for maintaining body turgor. This supports the hypothesis that this land crab species use a novel kind of skeletal support system, a "pneumo-hydrostat," during molting.

Finally, the external dimensions of the walking leg scales isometrically in hydrostatic and rigid animals of both crab species. Cuticle thickness, on the other hand, scales with isometry for rigid crabs of both species, but with negative allometry for hydrostatic crabs of both species. These results support the hypothesis that the two skeletal support mechanisms are affected by scale in different ways.

Future Directions

This dissertation research provides an initial description of the use of hydrostatic and pneumo-hydrostatic skeletal support in crabs. Several additional studies must be performed to understand fully how the arthropod body plan functions with a hydrostatic support mechanism and how this change in skeleton affects the physiology, behavior, and ecology of animals.

First, it is important to determine how muscle properties are affected by the switch to hydrostatic support. Muscle is an integral component of skeletal support systems; without it, movement would not be possible. During molting, some animals become noticeably sluggish and the forces of muscle contraction are significantly reduced (Chapters 2 and 4). But it is unclear if this results from changes in cuticle stiffness or changes in muscle properties. Muscle atrophy occurs in the propodus of the cheliped, but has not been reported previously for the other muscles (Mykles and Skinner, 1981). Muscle is a highly plastic tissue and how it adapts to a changing skeleton determines how well an animal functions during molting. To fully understand the biomechanics involved in switching skeletons, and how the skeletal

support system influences an animal's performance and behavior, potential changes in the structure and contractile properties of the muscle must be determined.

Second, it would be informative to model how the arthropod body plan functions as a hydrostat. The arthropod body plan evolved as a jointed, point-loaded lever system, with muscles inserting on stiff skeletal elements. Hydrostatic animals are mostly cylindrical in shape, with sheets of orthogonally arranged muscles surrounding a centralized volume of fluid. It is of interest to understand how bending at joints is accomplished in a hydrostatic crab and how muscle contracts against a soft cuticle to produce movement.

Third, a detailed morphological analysis should be made of newly molted crabs to determine how the pereopods are able to move independently. Preliminary analyses of the internal morphology of blue crabs revealed no obvious structures for partitioning and increased pressures are observed throughout the body during local muscle contraction (Chapter 2). The morphology of the muscles that pass through the coxae should be examined to determine if they potentially serve as sphincters and partition the body during movements. Furthermore, the distribution of water throughout the body, and any subsequent movement of water during muscle contraction, could potentially be determined using magnetic resonance imaging.

Fourth, scale effects on hydrostatic and rigid skeletons must be examined more comprehensively to draw definitive conclusions. This requires measurements of more individuals of *C. sapidus* and *G. lateralis* encompassing a broader size range. Furthermore, measuring a greater range of species, including unusually large species such as the gigantic Japanese Spider crab, would provide interesting and more broadly applicable conclusions.

Fifth, I would like to determine the effects of reduced cuticle stiffness on locomotion and anti-predator behavior in aquatic and terrestrial crabs. Surprisingly little is known about how molting animals avoid predation during these critical time periods. Common strategies to deter potential predators, such as the production of loud warning sounds, and the ability to escape predators may only function with a hard skeleton, but may be compromised with a soft, hydrostatic skeleton. Thus, arthropods may have evolved defensive strategies specific to this critical period and these strategies may differ in animals from different environments. It would, therefore, be important to determine how anti-predators strategies are modified as the skeleton changes during molting in aquatic and terrestrial crabs. This could be accomplished by determining if the rate of sound production and the acoustic characteristics and/or mechanisms change as the properties of the skeleton change, and also if locomotor ability and agonistic behaviors change during molting. Ultimately, it would be desireable to develop devices for tracking and monitoring molting crabs in the field to learn about molting behaviors and predation in the natural environment.

Finally, postmolt skeletal support mechanisms should be examined in other crustaceans and arthropods to understand molting adaptations in animals of various habitats and ecologies, such as crabs that live in the deep sea or in trees in the rainforest.

The tremendous diversity in body size, body shape, locomotor patterns, habitats, and ecologies of molting animals, along with their use of the two basic types of skeletons, make them ideal for studying how skeletal support systems evolve, correlate with the environment, and influence behavior. For future studies, I plan to examine skeletal support in other molting animals, locomotion and agonistic behaviors as a function of skeletal stiffness, and molting behavior in the natural environment. This unique approach will lead to important new

insights into the adaptations that arthropods have made to the risky process of molting, which is integral to their successful invasion of nearly every habitat on earth. Ultimately my research will provide insight into how skeletal support systems influence the way animals are built, develop, and function.

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