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Bite-Force Generation and Feeding Biomechanics in the Loggerhead Musk Turtle, Sternotherus Minor: Implications for the Ontogeny of Performance

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BITE-FORCE GENERATION AND FEEDING BIOMECHANICS IN THE LOGGERHEAD MUSK TURTLE, STERNOTHERUS MINOR: IMPLICATIONS FOR THE ONTOGENY OF PERFORMANCE

By

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For my parents,
Michael Pfaller and Beverly Ringenberg
and
my brother,
Stephen Pfaller
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Ontogenetic growth can profoundly affect the ability of organisms to perform ecologically-relevant feeding tasks that ultimately impact survival. In particular, bite-force generation is exceedingly important for vertebrates that process and consume robust prey (i.e. durophagy). Consequently, bite-force generation used in durophagy is a suitable parameter to investigate the functional relationships between musculoskeletal biomechanics, feeding performance, and ecology. I studied the ontogeny of bite-force generation and feeding biomechanics in the durophagous turtle, *Sternotherus minor*. Across an ontogenetic series of 75 *S. minor*, craniofacial growth was characterized by allometric increases (i.e. positive allometry) in the dimensions of the head and beak. Moreover, bite-force generation increased with positive allometry relative to body and head dimensions. These results indicate that ontogenetic modifications to the lever mechanics of the jaw system, and/or changes in the size (i.e. mass) and/or physiology (e.g., fiber lengths, degree of pennation) of the jaw adductor musculature have more explanatory power for bite-force generation than external measures.

A detailed, quantitative examination of the musculoskeletal biomechanics was performed to elucidate how these animals are capable of generating disproportionately high bite forces throughout ontogeny. Mechanical levers, muscle masses, and muscle architecture (fiber lengths and pennation angles) were measured from an ontogenetic series of 30 *S. minor*. With these data, a biomechanical model of the feeding apparatus was developed that accurately predicts individual and ontogenetic scaling of bite forces. Increasing muscle masses and changing the muscle architecture resulted in an increase in total physiological cross-sectional area ($P_{CSA}$) of the jaw adductor muscles that was proportional to changes in bite-force generation. These results indicate that the disproportionate increase in bite-force generation relative to skull length found in *S. minor* is explained by allometric changes in muscle size and architecture that collectively act to allometrically elevate the $P_{CSA}$ and muscle force. The alternative explanation of improving the mechanical leverage was not supported. Dietary data indicated that the positive allometry in bite-force generation observed in *S. minor* is tightly linked to the incorporation of
exponentially larger snails into the diet and positive allometry of the forces required to fracture the largest dietary items. These forces were found to be greater than the observed and theoretical bite forces, which suggested that fatigue failure resulting from multiple bite-force loadings may allow *S. minor* to fracture snails at lower compressive forces and access large snails that are apparently outside the range of their bite-force capacity. Moreover, age-based growth patterns for bite-force generation fit a logistic growth curve and reveal a close relationship with the forces required to fracture snails found in the diet. The results of this study provide empirical evidence that ontogenetic changes to musculoskeletal morphology and ecology are inextricably linked by the performance of the feeding apparatus in *S. minor*. Such results are exceedingly important for establishing a baseline from which to explore the mechanistic and functional issues that underlie the evolution of phenotypic traits among vertebrates.
INTRODUCTION

All vertebrates increase in size during development. The consequences of such changes for organismal design have long been of interest to biologists (e.g., Huxley 1924, 1932; Hill 1950; Gould 1966, 1971; Schmidt-Nielsen 1984). In particular, since changes in size necessitate changes in functional ability (Hill 1950; Schmidt-Nielsen 1984), the implications of growth for ecological performance and the occupation of niches have received considerable attention (e.g., Marsh 1988; Carrier 1996; Binder and Van Valkenburgh 2000; Hernández 2000; Erickson et al. 2003; Christiansen and Adolfsen 2005; Gibb et al. 2006; Herrel and O’Reilly 2006; Brecko et al. 2008). The ability of vertebrates to perform ecologically-relevant tasks during growth is tightly linked to the morphology of the musculoskeletal system and ultimately determines patterns of resource use by organisms (e.g., Kiltie 1982; Wainwright 1987; Hernández and Motta 1997; Husak et al. 2006; Bulté et al. 2008). Consequently, understanding how variation in musculoskeletal growth leads to differences in functional ability (i.e. performance) has become a fundamental goal in fields of functional morphology and biomechanics (e.g., Arnold 1983; Wainwright 1987; Kingsolver and Huey 2003).

The vertebrate skull and associated musculature is a hallmark form-function complex (Anderson et al. 2008), in which much of the vast diversity among species reflects features related to feeding (Smith 1993). Feeding is of paramount importance during the ontogeny of all animals, as the energy acquired during feeding fuels subsequent growth and reproduction. The performance of the feeding apparatus, therefore, has profound impacts on survival through its role in procuring, processing, and ingesting food resources (Emerson and Bramble 1993). Consequently, the implications of ontogenetic growth for the scaling of feeding structures and their influence on feeding performance have been studied in a broad range of taxa (e.g., Hernández and Motta 1997; Binder and Van Valkenburgh 2000; Hernández 2000; Erickson et al. 2003; Herrel and O’Reilly 2006; Huber et al. 2006, 2008). While there is a suite of potentially relevant performance measures associated with the vertebrate feeding system (e.g., suction performance or velocity of jaw-prehension), bite-force generation has been shown empirically to
be an important determinant of ontogenetic changes in niche use and breadth (e.g., Wainwright 1987; Herrel et al. 1996, 1999, 2001; Hernández and Motta 1997; Erickson et al. 2003; Grubich 2003, 2005; Herrel and O’Reilly 2006; Brecko et al. 2008; Bulté et al. 2008). The capacity to generate bite forces directly influences whole-animal performance (Anderson et al. 2008), therefore variation among individuals in bite-force generation may be linked to variation in fitness (Wainwright 1991).

In particular, the utility of bite-force generation can be most obviously observed in organisms that process and consume durable prey items (e.g., bone, mollusks, seeds), a foraging mode known as durophagy. Durophagy has been described in virtually all vertebrate groups [chondrichthyans (e.g., Summers 2000; Wilga and Motta 2000; Huber et al. 2005, 2008), osteichthyans (e.g., Wainwright 1987; Turingan and Wainwright 1993; Hernandez and Motta 1997; Grubich 2003, 2005), amphibians (Inger 1966), squamates (e.g., Dalrymple 1979a; Pregill 1984; Wineski and Gans 1984; Green et al. 1997; Jackson and Fritts 2004), turtles (e.g., Carr 1952; Legler and Georges 1993; Bonin et al. 2008), crocodilians (e.g., Dodson 1975; Cleuren and De Vree 2000; Brochu 2001), birds (e.g., van der Meij and Bout 2004; Herrel et al. 2005a), and mammals (e.g., Kiltie 1982; Werdelin 1989)]. Durophagy is almost invariably associated with an ontogenetic increase in bite-force generation that allows consumers to expand their diet (e.g., Wainwright 1991; Binder and Van Valkenburgh 2000) or specialize into a niche where there are few competitors (Wainwright 1987; Summers 2000; Herrel et al. 2002; Bulté et al. 2008). In the case of durophagy, because maximum bite-force generation is ecologically relevant and is most likely under strong selection for efficient design and performance, it is a good parameter from which to examine the relationship between ontogenetic growth and performance.

Ontogenetic (or phylogenetic) increases in maximum bite force can be achieved by simply growing larger, as a direct scaling consequence of becoming larger is an absolute increase in muscle-force generation (Hill 1950; Schmidt-Nielson 1984). As a result, maximum bite-force generation has been found to be strongly correlated with overall body size in all taxa studied (Anderson et al. 2008; Pfaller see Chapter 1). Along the same lines, ontogenetic increases in the dimensions of the head (e.g., length, width, depth) in the absence of shifts in muscle physiology, lever mechanics, or changes in size, will also increase bite forces. All available data, however, from a broad range of taxa show that bite-force generation increases disproportionately relative
to changes in head and body dimensions (see review in Herrel and Gibb 2006; Pfaller see Chapter 1). These results suggest that there is likely differential scaling of musculoskeletal traits related to biomechanics of bite-force generation. In the absence of changes in size, biomechanical theory predicts that the force generating capacity of a musculoskeletal apparatus may be increased by improving the mechanical leverage of the system and/or by increasing muscle-force generation (Cochran 1982). Since muscle-force generation is a function of the physiological cross-sectional area of the muscle generating the force (Emerson and Bramble 1993), it can be elevated by increasing the mass of the muscle or by changing the fiber architecture (i.e. the degree and angle of pennation, fiber length; Gans and De Vree 1987). Consequently, the scaling of these traits likely has more explanatory power in terms of ontogenetic changes in bite-force generation than external head and body measures (Herrel et al. 2002; Pfaller see Chapter 1). Differential scaling of muscle mass and architecture, and lever mechanics, have been posited to explain the disproportionate increase in bite force, however relatively few detailed, quantitative studies have examined the musculoskeletal biomechanics of an ontogenetic series to support this observation. Furthermore, those that have are restricted to fishes (Wainwright 1987; Hernández and Motta 1997; Huber and Motta 2004; Grubich 2005; Herrel et al. 2005b; Huber et al. 2006, 2008).

Although studies of the ontogeny of bite-force generation in tetrapods are numerous (see review in Herrel and Gibb 2006), most investigations have scaled bite forces to external head measures, and speculated how these measures might relate to musculoskeletal feeding biomechanics (except see Thompson et al. 2003). Consequently, we know a great deal about the ecological importance of bite-force generation throughout development in tetrapods, but little in terms of how musculoskeletal growth makes this change possible. In addition, what limited work that has been done on the ontogeny of musculoskeletal biomechanics in tetrapods is from captive individuals (e.g., Thompson et al. 2003; Huhov et al. 2005), and work by Erickson and colleagues (2004) suggests that captivity can lead to significant biomechanical differences in performance that may confound inferences of natural performance. For these reasons, I sought to investigate the ontogenetic scaling of bite-force generation and feeding biomechanics in a wild population of durophagous tetrapods. Because durophy is relatively common in tetrapods (see citations above) and invariably involves a relative increase in bite-force generation, the results of
this study may serve as a model for how ontogenetic changes in morphology and ecology are
linked by the performance of the feeding apparatus in durophagous vertebrates in general.

The loggerhead musk turtle, *Sternotherus minor*, is a particularly suitable organism for
these purposes. These turtles undergo dramatic morphological changes, in which adults develop
hypertrophied skulls and triturating surfaces (Carr 1952; Tinkle 1958). These changes
correspond with developmental preference for more durable prey (Ernst *et al.* 1994; Zappalorti
and Iverson 2006). Juveniles have catholic diets that may include insects, millipedes, spiders,
crayfish, snails, clams, and fish (Tinkle 1958; Ashton and Ashton 1985), whereas adults
specialize on larger snails and clams (Carr 1952; Tinkle 1958). Because snails and clams are
present in the diets of both adults and juveniles, and likely represent the most durable prey,
maximum bite-force generation is likely directly related to the forces required to crush these prey
items. Consequently, it is reasonable to infer that bite-force generation is ecologically relevant in
this capacity and likely increases considerably across ontogeny as these animals process and
consume increasingly more durable prey.

Additionally, the feeding apparatus found in all turtles provides a simple, yet relevant,
system that facilitates the development of a biomechanical model for bite-force generation that
could be used as a tool to investigate hypotheses into how *S. minor* achieves disproportionately
high bite-force generation across ontogeny. First, the skulls of turtles are akinetic and
edentulous, thus the complexities associated with intracranial joints (aside from the jaw joint)
and dental morphology are not involved. Second, the adduction of the arcilineal lower jaw does
not show significant propalineal movements during prey processing (Reilly *et al.* 2001), and the
angle of bite forces are likely close to perpendicular (Sinclair and Alexander 1987). Finally, the
cranial musculature in turtles has been well described (Schumacher 1973), and is particularly
simple with a considerable portion of the muscle mass being held in a single muscle, the M.
adductor mandibulae externus. This muscle is typically bipennate (or multipennate) in turtles
(Schumacher 1973), thus allowing for an assessment into the role of pennation in bite-force
generation. The development of a biomechanical model of bite-force generation is important
because it provides testable hypotheses of the scaling relationships between the musculoskeletal
morphology and their effect on performance (Westneat 2003, 2004), and allows for the
identification of morphological traits that are particularly important for musculoskeletal function
(Scott and Winter 1991; Weishampel 1993).
My overall objective for this research was to evaluate the functional relationships between the ontogeny of feeding biomechanics, bite-force generation, and durophagy in *S. minor* with the intention of using these animals as a model for vertebrates with similar dietary specialization. To elucidate these relationships, my specific research objectives were to (1) quantify the degree of craniofacial hypertrophy in *S. minor* (Chapter 1), (2) confirm that bite-force generation scales disproportionately relative to head and body dimensions in *S. minor* as it does in other taxa (Chapter 1), (3) develop a biomechanical model for bite-force generation and test its accuracy by comparing theoretical bite forces with observed bite forces (Chapter 2), (4) determine the scaling relationships between biomechanical variables and bite-force generation to explain the disproportionate increase in bite-force generation displayed by *S. minor* (Chapter 2), (5) place bite-force generation in the context of the dietary ontogeny of *S. minor* by estimating the force required to fracture snails found in diet (Chapter 2), and (6) evaluate scaling relationships in terms of longevity using skeletochronology (Chapter 3).
CHAPTER 1

SCALING OF CRANIOFACIAL MORPHOLGY AND BITE-FORCE GENERATION IN THE LOGGERHEAD MUSK TURTLE, *STERNOTHERUS MINOR*

All vertebrates increase in size during development, and the consequences of changes in size for organismal design have long been of interest to biologists (e.g., Huxley 1924, 1932; Hill 1950; Gould 1966, 1971; Schmidt-Nielsen 1984). Changes in size necessitate changes in physical capacities (Hill 1950; Schmidt-Nielsen 1984). Hence the implications of growth for ecological performance and the occupation of niches have received considerable attention (e.g., Marsh 1988; Carrier 1996; Wainwright 1987; Binder and Van Valkenburgh 2000; Hernández 2000; Erickson *et al.* 2003; Christiansen and Adolfsson 2005; Gibb *et al.* 2006; Herrel and O’Reilly 2006; Brecko *et al.* 2008). The performance of the feeding apparatus, in particular, has profound impacts on survival through its role in procuring, processing, and ingesting food resources (Emerson and Bramble 1993), therefore the application of optimality criteria and engineering principles in analyses of allometric growth can elucidate possible adaptations to ecological problems and the potential for trade-offs and constraints (Schwenk 2000).

The bite forces invoked through the jaw adductor musculature and their mechanical leverage are particularly important for organisms that process and consume robust prey items (e.g., bone, mollusks, seeds), a foraging mode known as durophagy. Durophagy has been described in virtually all vertebrate groups [chondrichthyans (e.g., Summers 2000; Wilga and Motta 2000; Huber *et al.* 2005, 2008), osteichthyans (e.g., Wainwright 1987; Hernandez and Motta 1997; Grubich 2003, 2005), amphibians (Inger 1966), squamates (e.g., Dalrymple 1979a; Pregill 1984; Wineski and Gans 1984; Green *et al.* 1997; Jackson and Fritts 2004), turtles (e.g., Carr 1952; Legler and Georges 1993; Bonin *et al.* 2008), crocodilians (e.g., Dodson 1975; Cleuren and De Vree 2000; Brochu 2001), birds (e.g., van der Meij and Bout 2004; Herrel *et al.* 2005a), and mammals (e.g., Kiltie 1982; Werdelin 1989)]. Durophagy is typically associated...
with morphological specializations that allow consumers to inhabit a niche where there are few competitors (Wainwright 1987; Bulté et al. 2008). For these reasons, bite-force generation is ecologically relevant and is most likely under strong selection for efficient design and performance, therefore it is a suitable parameter from which to examine the relationship between growth and biomechanical performance.

The edentulous, akinetic feeding apparatus found in turtles provides a simple, yet relevant, system to conduct an investigation of the ontogeny of feeding performance. In addition, feeding mode has been found to be a key factor determining morphological evolution and diversification of turtles, whereby diet (especially durophagy) leads to comparable morphologies in different clades (Claude et al. 2004). While considerable attention has been paid to the organization of skull elements and musculature in turtles (e.g. Schumacher 1973; Gaffney 1979), limited data have been obtained on the biomechanical performance of the jaws (e.g. bite force; see Herrel et al. 2002; Herrel and O’Reilly 2006; Bulté et al. 2008) and rhamphotheca (e.g. strength, pressure generation). In the present study, I examine the role of growth in the scaling of bite-force generation in an ontogenetic series of wild loggerhead musk turtles, *Sternotherus minor*. It is well established that throughout the majority of their range dramatic morphological changes occur whereby adults develop hypertrophied skulls and jaw musculature, and expanded triturating surfaces (Carr 1952; Tinkle 1958). These changes allow increased durophagy across ontogeny (Ernst et al. 1994; Zappalorti and Iverson 2006). Juveniles have been shown to feed on a variety of prey items, such as insects, millipedes, spiders, crayfish, snails, clams, and fish (Ashton and Ashton 1985; Tinkle 1958), while adults primarily consume snails and clams (Carr 1952; Tinkle 1958). These more durable food items can be found in high densities (> 300 per m$^2$; Berry 1975) and occasionally represent a large proportion of the adult diet (> 85%; Berry 1975). For these reasons, *S. minor* is a particularly suitable taxon to examine the scaling of morphology and bite-force generation in a population of wild turtles.

For this work, bite forces were recorded throughout ontogeny in a complete growth series of *S. minor* using a set of precision, electronic bite-force transducers. In addition, external measurements of the body, head, and beak were made in each specimen. I determined how the scaling of bite-force generation compares to the scaling of body, head, and beak measures. I used a simple model of geometric similarity (i.e., isometry) as a null hypothesis against which I compared empirically determined coefficients of scaling (Hill 1950; Emerson and Bramble
The geometric similarity model is based on Euclidean geometry and predicts that as an object grows in linear dimensions by \( n \), its area measures increase by \( n^2 \) and volume (or mass) by \( n^3 \) (Hill 1950; Schmidt-Nielson 1984; Emerson and Bramble 1993). Because muscle-force generation is predicted to scale in proportion to the total cross-sectional area of the muscles involved, bite-force generation, if isometry is assumed and performance is at the maximum, will scale to the square of linear measurements (logarithmic slope = 2.0) and the two-thirds power (logarithmic slope = 0.67) of body mass (Hill 1950; Schmidt-Nielson 1984). In addition, the scaling coefficient based on geometric similarity for a comparison of two linear measures is predicted to have a logarithmic slope of 1.0 (Hill 1950). Because significant deviation from geometric scaling (i.e. allometry) may be biologically meaningful, I then discuss these results in the context of feeding behavior and biomechanical performance.

Objectives of this research included: (1) quantifying the ontogenetic changes in craniofacial morphology in \( S. \ minor \); (2) determining how bite-force generation scales relative to external morphological changes; and (3) evaluating the relationship between ontogenetic growth, bite-force generation, and dietary ontogeny.

**Materials and Methods**

**Study Site and Specimens**

To assess scaling of morphology and bite-force generation in wild turtles, this study required an entire growth series of a species that displays morphological modifications that reflect an ecologically relevant increase in bite-force generation during development. The loggerhead musk turtle, \( S. \ minor \), is a suitable taxon for these purposes and was accessible for testing in large numbers along the Rainbow River, Marion Co., Florida, USA. Although not historically common (Marchand 1942), the Rainbow River now supports large numbers of \( S. \ minor \) (up to 127 per ha; Meylan *et al.* 1992) and has been a site for previous research on population dynamics, growth rates, and age distribution (Meylan *et al.* 1992; Onorato 1996). The Rainbow River is a 5.7 km spring run between Rainbow Spring and the Withlachoochee River, and represents the southern extent of the range for \( S. \ minor \). The spring fed nature of the Rainbow River results in nearly constant year-round temperature (~72°), and
allows *S. minor* to be active throughout the year. Moreover, slow currents, wide banks, and large patches of submerged vegetation are present and provide turtles with favorable sites for foraging and refuge.

In the spring of 2008, a total of 284 *S. minor* were captured by hand via snorkeling. Seventy-five specimens spanning development were selected for bite-force experimentation and morphometric analysis (36.9 – 134.1 mm carapace length). Specimens were held in shaded containers prior to data collection and released near the location of capture within 8 h of being removed. Specimens that appeared to have sustained minor facial or beak trauma resulting from prior agonistic interactions were not selected for bite-force experimentation.

**Morphology**

Whole body, head, and beak measures were taken from each individual (Figure 1.1). Body size measures included: body mass (M) and carapace length (CL; notch to notch). Four major osteological and myological features of the head were measured to quantify head size and shape (Figure 1.1): head length from the anterior edge of the premaxilla to the posterior tip of the supraoccipital blade (HL), head width across the tympanic region (HW), head depth across the tympanic region (HD), and head circumference measured in the frontal plane around the tympanic region (HC; combined measure of HD and HW). In addition, seven measurements were taken to quantify beak shape (Figure 1.1): upper beak length (UBL), upper beak width (UBW), upper beak depth (UBD; taken at the mid-point just anterior to the eye), lower beak length (LBL), lower beak width (LBW), lower beak depth (LBD), and symphysis length (SL). Linear measurements were made with digital calipers to the nearest 0.01 mm, head circumference was measured to the nearest 1.0 mm with a flexible measuring tape, and mass was measured to the nearest 1.0 g on a digital scale.

**Bite Force Testing Protocol**

*Equipment.* Testing bite-force generation throughout ontogeny in *S. minor* required kinematically comparable, maximal bite force values for animals ranging from hatchlings (5-10 g) to larger adults (350-360 g). To meet these specifications, a miniaturized precision force transducer with two replaceable bite-plate configurations was constructed (Figure 1.2). This device was constructed with a high impedance load cell (Kistler Instrument Corp., Type 9212,
Amherst, NY) sandwiched between two stainless-steel cantilever beams, such that the voltage output routed through a charge amplifier (Kistler Instrument Corp., Type 5995A, Amherst, NY) was proportional to the compressive force applied to dual cantilever beams. For animals less than 100 g (~ < 88 mm carapace length) with expected low bite-force magnitudes and small gape spans, a set of bite plates of 1 mm thickness configured 1 mm apart (total gape = 3 mm) were used. For larger animals greater than 100 g (~ > 88 mm carapace length), a set of bite plates of 1.5 mm thickness configured 2 mm apart (total gape = 5 mm) were used. The bite plates and cantilever beams were machined from stainless steel and aluminum, respectively. Flat strips of compressive rubber were placed between the bite plates to prevent contact under high loading. Leather strips were affixed to the end of the biting surface of each beam as points of contact for the upper and lower beak of the animals. The leather served to lower the strain-rate density of the beak during tests, thus eliminating the potential for trauma. In addition, they provided a fixed point for beak contact, which eliminated error resulting from variation in bite position along the length of the bite plates (a known issue of cantilever-based bite bars; McBrayer and White 2002; Erickson et al. 2003), and allowed me to obtain repeatable bite-force measurements.

The bite-force apparatus was calibrated by statically suspending a series of ascending weights (spanning the range of expected bite forces) at the bite point (Dechow and Carlson 1983). The mass of each weight and the force readout registered by the charge amplifier were recorded and plotted. Trials consistently produced a linear relationship for both bite-plate configurations (small, $r^2 = 0.989$, $n = 7$; large, $r^2 = 0.991$, $n = 7$). The transducer was calibrated before and after bite-force trials, and proved to be highly stable.

**Bite-force trials.** Animals were restrained, and appropriate bite plates were placed unilaterally between the jaws, centered mesio-distally at the trough of the crushing surface along the lower beak. This site is directly lateral to the posterior limit of the mid-sagittal jaw symphysis, and represents the location along the triturating surface that *S. minor* primarily processes snails (Pfaller personal observation). To obtain bite forces at comparable gape angles for the very smallest individuals (< 40 g; $n = 8$), bite-force generation was measured at the anterior tips of the beak with the small bite-plate configuration, then converted to an equivalent, more posterior bite point based on lever mechanics. *Sternotherus minor* of all sizes readily gape their mouths when threatened; if necessary, animals were encouraged to gape with light taps on
the snout. The presence of the bite plates between the jaws typically elicited repeated, aggressive bites. Each individual was measured for subaerial bite-force generation during a sequence of three bite-force trials separated by approximately two minutes. The highest of the 3 bite forces registered by an individual was treated in post hoc analyses as the maximum bite force.

**Statistical Analysis**

Raw morphometric data were plotted against standard measures of head and body size, and raw bite-force data were plotted against each of the raw morphometric variables. Except for calculations of descriptive statistics, all data were then logarithmically transformed (log$_{10}$) for further analysis. To assess scaling among morphometric variables, reduced major axis regressions were performed with morphometric indices of body size (carapace length; CL) and head size (head length; HL) as independent variables and other morphometrics as the dependent variables. Carapace length was selected as the index of body size because it is a standard measure of body size in chelonian studies, and HL was selected as the index for head size (Bever 2008). Similarly, to assess scaling of bite-force generation, reduced major axis regressions were performed with morphometrics as the independent variables and bite-force generation as the dependent variable. Reduced major axis regressions on log-transformed data yielded equations in the form:

$$\log y = \log a + b \log x,$$

in which $x$ is the independent variable, $y$ is the dependent variable, $a$ is the $y$ intercept, and $b$ is the scaling or regression coefficient (Sokal and Rohlf 2000). The scaling coefficient describes the relative allometry or isometry of the relationship. Deviations from isometry (i.e., allometry) were considered significant if the appropriate predicted slopes based on isometry (= “geometric similarity”; Schmidt-Nielson 1984) fell outside of the 95% confidence intervals of the observed slopes. Scaling coefficients significantly greater or less than that predicted by isometry were designated as positive or negative allometry, respectively. Reduced major axis regressions were plotted with the appropriate predicted linear relationship superimposed on each plot. Ontogenetic changes in morphology and bite-force generation were also analyzed against published information on the dietary ontogeny of *S. minor*. 

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The effect of sex was determined using a series of unpaired \( t \)-tests and one-way analyses of covariance (ANCOVA). Unpaired \( t \)-tests and analyses of covariance were performed for sexually mature adults only (specimens \( \geq 80 \) mm CL; Iverson 1978; Etchberger and Ehrhart 1987). To remove the effect of body size, CL was used as a covariate. Statistical analyses were done using R for Windows v. 2.8.1 (The R Foundation for Statistical Computing, 2008).

**Results**

**Scaling of Morphology**

Descriptive statistics are shown in Table 1.1. All measures of the head (HL, HW, HD, HC) were significantly and highly correlated with carapace length (CL) (Table 1.2). All measures of the head (HL, HW, HD, HC) scaled to CL showed coefficients (slopes) significantly greater than the logarithmic value of 1.0 predicted by isometry (positive allometry) (Table 1.2; Figure 1.3). Within the cranium, all measures of the head (HW, HD, HC) and beak (UBL, UBW, UBD, LBL, LBW, LBH, LBD, SL) were significantly and highly correlated with head length (HL) (Table 1.2). All measures of the head (HW, HD, HC) and some of the beak (UBD, UBW, LBW, LBD, SL) scaled to head length showed coefficients significantly greater than the isometric value of 1.0 (Table 1.2; Figures 1.4, 1.5). Two measures of the beak (UBL, LBL) scaled to head length showed coefficients not significantly different from the value of 1.0 predicted by isometry (Table 1.2; Figures 1.5a, b).

**Scaling of Bite-Force Generation**

Bite-force generation was significantly and highly correlated with all morphological variables that were considered (Table 1.2). Bite-force regressions scaled to body size (CL), head size measures (HL, HW, HD, HC) and some beak measures (UBL, UBW, UBD, LBL, LBW) showed coefficients significantly greater than the logarithmic value of 2.0 (positive allometry) (Table 1.2; Figures 1.6a, 1.7, and 1.8a, b). Bite-force regressions scaled to other beak measures showed coefficients significantly less than the predicted value of 2.0 (negative allometry; LBD, SL) (Table 1.2; Figure 1.8c). Bite-force regressions scaled to body mass showed coefficients
significantly greater than the logarithmic value of 0.67 predicted by isometry (positive allometry) (Table 1.2; Figure 1.6b).

Differences Between the Sexes

Among adult individuals (CL > 80 mm; n = 58), males were significantly larger than females for most morphometric variables (all except carapace length; t = 1.86, P = 0.07) and for absolute bite force (t = 2.452, P = 0.018; Table 1.1). However, with the effect of size removed, all morphometric variables and bite-force generation (F = 0.31, P = 0.58) did not differ between adult males and females (Table 1.1). These results corroborate similar findings by Tinkle (1958) and Berry (1975), and justified the pooling of males and females in the other analyses.

Discussion

Relationship Between Morphology and Bite-Force Generation

The goal of the present study was to elucidate the linkages connecting ontogenetic changes in morphology with variation in a proxy for ecological performance (bite-force generation) by comparing empirically determined coefficients of scaling with isometric predictions. I found that ontogenetic changes in overall size in *Sternotherus minor* were highly correlated with changes in craniofacial morphology and bite-force generation. These correlations, however, rarely conformed to predictions based on isometric growth. Instead, ontogenetic growth in *S. minor* was characterized by allometric increases (i.e. positive allometry) in the dimensions of the head and beak relative to indices of body (carapace length) and head size (head length). In addition, because muscle-force generation is predicted to scale in proportion to the total cross-sectional area of the muscles involved, bite-force generation was predicted to scale to the square of linear measurements (logarithmic slope = 2.0) and the two-thirds power (logarithmic slope = 0.67) of body mass (Hill 1950; Schmidt-Nielson 1984). Results, however, indicate that bite-force generation did not scale to body or head size, or beak measures, in the proportions predicted from isometric growth. Instead, bite-force generation scaled with positive allometry relative to nearly all morphological measurements.
That all measures of the body, head, and beak were significantly and highly correlated with bite-force generation in *S. minor* suggests that overall changes in body size across ontogeny are important for increasing absolute bite-force generation. This is not surprising, as maximum bite force has been found to be strongly correlated with overall size in all taxa studied (Anderson *et al.* 2008). That larger animals tend to bite more forcefully than smaller animals is a direct consequence of the scaling of muscle forces relative to linear dimensions (Hill 1950; Schmidt-Nielsen 1984). Consequently, for many species that require an increase in absolute bite force to successfully compete for resources and mates as adults, one option is to simply grow larger. Nevertheless, because bite-force generation in *S. minor* scaled with significant positive allometry relative to changes in body size (carapace length and mass), such changes only partially explain the ontogenetic increase in bite-force generation.

In addition to increasing overall body size, an ontogenetic increase in bite-force generation may also be achieved by disproportionate increases in head size and shape (Herrel *et al.* 2002). In *S. minor*, all measures of head size (length, width, depth, and circumference) scale with positive allometry relative to carapace length. These results stand in contrast to those of Herrel and O’Reilly (2006), who found in three different species of turtles (*Chelydra serpentina*, *Staurotypus* sp., and *Trachemys scripta*) that most measures of head size scale with negative allometry relative to carapace length (except head width in *C. serpentina* and head length in *Staurotypus* sp. which scaled isometrically). Additionally, Lindeman (2000) and Bulté *et al.* (2008) found that the “megacephalic,” durophagous turtles of the genus *Graptemys* also display either isometry or negative allometry in head size (width) relative to body size (plastron length). The presence of either negative allometry or isometry in head dimensions relative to body size is an almost universal trait among vertebrates (e.g., Radinsky 1981; Meyers *et al.* 2002; Herrel *et al.* 2005a; Herrel and O’Reilly 2006), therefore to my knowledge *S. minor* is one of the only vertebrate taxa that displays positive allometry in head length, width, and depth relative to body size. As a consequence, these deviations from isometry in head dimensions displayed by *S. minor* are almost certainly biologically meaningful in terms of the ontogenetic changes in foraging behavior (i.e., bite-force generation used in durophagy).

Despite these unique scaling relationships, however, bite-force generation in *S. minor* still scales with positive allometry relative to changes in head size (length, width, depth, and circumference). Similar results were found by Herrel and O’Reilly (2006) for *C. serpentina*
(head width and height) and *T. scripta* (head length, width, and height), and by Bulté *et al.* (2008) for male *G. geographica* (head width). While changes to head size did not scale isometrically with bite force in *S. minor*, such changes are likely important and can be discussed in reference to their influence on bite-force generation (Emerson and Bramble 1993). Positive allometry in head dimensions relative to carapace and head length in *S. minor* likely relates to ontogenetic modifications to the skull related to the accommodation of hypertrophied jaw adductor musculature (Pfaller see Chapter 2). Because the M. adductor mandibulae externus (the largest adductor muscle in turtles; Schumacher 1973) turns posteriad at the trochlear process and attaches along the supraoccipital and parietal bones medially and posteriorly, and the quadrate and squamosal laterally (Schumacher 1973), a lengthening, deepening, and widening of the skull would provide added surface area for attachment of a distinctly larger muscle mass. Corroborating this theory, Dalrymple (1977) found that male and female *Apalone (=Trionyx) ferox* display significant positive allometry in skull width and adductor muscle mass (M. externus and M. zygomaticomandibularis) relative to basicranial skull length. Not surprisingly, jaw adductor muscle masses have been shown to be strong predictors of bite-force generation in multiple taxa (e.g., Hernández and Motta 1997; van der Meij and Bout 2004; Herrel *et al.* 2008). Moreover, Herrel *et al.* (2002) and Herrel and O’Reilly (2006) suggest that in turtles head widening and deepening may result in a more favorable orientation of the jaw adductors relative to the lower jaw, in which a larger proportion of the muscle force acts parasagittally.

As previously mentioned, ontogenetic changes to body size and head dimensions only partially explain the observed allometric increase in bite-force generation in *S. minor*: bite-force generation increases with positive allometry relative to all head measures. Similar departures from isometry in bite-force generation relative to body and head dimensions have been documented in a broad range of vertebrate taxa: fishes (Hernández and Motta 1997; Herrel *et al.* 2005b; Huber *et al.* 2006, 2008), lizards (e.g., Herrel *et al.* 2001; Meyers *et al.* 2002; Herrel and O’Reilly 2006), turtles (Herrel and O’Reilly 2006; Bulté *et al.* 2008), crocodilians (Erickson *et al.* 2003), birds (van der Meij and Bout 2004; Herrel *et al.* 2005a), and mammals (Binder and Van Valkenburgh 2000). Perhaps not surprisingly, these data suggest that ontogenetic modifications to the lever mechanics of the jaw system, and/or changes in the size (i.e. mass) and/or physiology (e.g., fiber lengths, degree of pennation, activation patterns) of the jaw...
adductor musculature may have more explanatory power for bite-force generation than external measures (Herrel et al. 2002; Pfaller see Chapter 2).

Ontogenetic modifications to beak morphology in *S. minor* showed a general trend toward widening and deepening: all measures of beak (upper beak width, lower beak width, upper beak depth, lower beak depth, symphysis length) scaled with positive allometry relative to head length with the exceptions being, not surprisingly, the length of the upper and lower beak which scaled isometrically. Allometric increases in beak width and depth likely correspond to equivalent changes in the width and depth of the head; however, such changes to the beak may also be important for resisting reaction forces incurred during high load situations (Herrel et al. 2005a). Our data indicate that two measures, lower beak depth and symphysis length, had scaling coefficients dramatically greater than the isometric scaling predictions, indicating that these traits are particularly important. Similar results were found by Dalrymple (1977) and Bever (2008) for both male and female *Apalone (=Trionyx) ferox* and *Pseudemys texana*, respectively. In both studies, mandibular width and symphysis length scaled with positive allometry relative to skull length [basicranial skull length in Dalrymple (1977)]. These measures in *S. minor* were also the only two morphometrics for which bite-force generation scaled with negative allometry (for all other head and beak measures, bite-force scaled with positive allometry). These negatively allometric relationships are a result of the lower jaw and mandibular symphysis becoming disproportionately more robust. Lower beak depth is a function of the girth of the mandible and likely facilitates force transfer around the mandible by resisting bending deformation during typical loading situations. In addition, mandibular symphyseal biomechanics have been studied extensively by mammalogists (e.g., Hylander 1985; Greaves 1988), and results indicate that a lengthening of the mandibular symphysis will aid in resisting dorsoventral symphyseal shear and transverse symphyseal torsion resulting from bilateral contraction of the adductor muscles during unilateral biting (Witmer and Rose 1991). Further studies quantifying mandibular and symphyseal stresses during high bite-force generation are needed to test the validity of these hypotheses in turtles.

**Implications for Dietary Ontogeny**
The ecological implications for positive allometry in head size, beak shape, and bite-force generation are likely linked to significant changes in the types and physical attributes of *S. minor* prey during ontogeny. Juvenile *S. minor* (< ~ 80 mm carapace length) consume a wide variety of food items, including insects, millipedes, spiders, earthworms, crayfish, snails, clams, fish, carrion, aquatic plants, and algae. Conversely, adults process and consume mostly larger snails and clams (Carr 1952; Tinkle 1958; Berry 1975; Ernst *et al.* 1994; Pfaller see Chapter 2). In some populations, the particularly thick-shelled snails (Gastropoda) of the genus *Goniobasis* (=*Elimia*) constitute more than 85% (by volume) of the dietary contents in adult *S. minor* (Berry 1975). Specialization on such durable prey requires a feeding apparatus with sufficient biomechanical properties to generate and support high bite forces, and likely allows *S. minor* to occupy a nearly competitor free niche (Kiltie 1982; Herrel *et al.* 2002; Anderson *et al.* 2008). In addition, studies by Osenberg and Mittelbach (1989), Hernández and Motta (1997), and Tucker (1997) have found that the crushing resistance strength of many gastropod mollusks increases disproportionately with changes in size. The incorporation of more and larger durable prey into the diet by larger *S. minor* (Berry 1975; Pfaller see Chapter 2), therefore, likely necessitates the allometric changes in craniofacial morphology and bite-force generation found in this study. More work is needed to identify the relationship between the morphology and bite-force generation in *S. minor*, and the crushing-resistance strength of their molluscan prey (Pfaller see Chapter 2). Such an investigation would reveal whether *S. minor* is trophically limited across ontogeny by bite-force generation and therefore constrained by the structural resistance of its prey (Wainwright 1987; Meyer 1989; Mittelbach *et al.* 1992; Hernández and Motta 1997).
Figure 1.1. Skull of the loggerhead musk turtle, *Sternotherus minor*, illustrating measurements. 1-2, head length (HL); 3-4, head width (HW); 5-6, head depth (HD; across tympanic region); 8-9, upper beak length (UBL); 10-11, upper beak depth (UBD); 7-12, lower beak length (LBL); 13-14, lower beak depth (LBD); 7-15, symphysis length (SL); 16-17, upper beak width (UBW); 18-19, lower beak width (LBW); 20, head circumference (HC; frontal plane).
Figure 1.2. Schematics of the miniaturized precision force transducer with two replaceable bite-plate configurations used to measure bite-force generation in *Sternotherus minor*. The small bite-plate configuration was used when testing animals less than 100 g (~ < 88 mm carapace length) and the large bite-plate configuration was used when testing animals greater than 100 g (~ > 88 mm carapace length).
Table 1.1. Summary statistics for raw morphological and bite-force data, and results of unpaired t-tests and one-way analysis of covariance. Unpaired t-tests and analysis of covariance (ANCOVA) were done to compare morphological variables and bite-force generation between adult male and female *Sternotherus minor*. For the ANCOVA, carapace length was used as the covariate. All data were logarithmically transformed for t-tests and ANCOVA.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Immature ( (n = 17) ) (mean ± SE)</th>
<th>Adult ( \varphi ) ( (n = 24) ) (mean ± SE)</th>
<th>Adult ( \delta ) ( (n = 34) ) (mean ± SE)</th>
<th>Unpaired t-test ( (n = 75) )</th>
<th>ANCOVA ( (n = 75) )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>t-value ( P )</td>
<td>F-value ( F ) ( P )</td>
</tr>
<tr>
<td>CL (mm)</td>
<td>62.7 ± 13.1</td>
<td>103.3 ± 15.9</td>
<td>110.8 ± 14.9</td>
<td>1.86 0.070</td>
<td>1.79 0.07</td>
</tr>
<tr>
<td>Mass (g)</td>
<td>45.5 ± 24.8</td>
<td>180.7 ± 80.9</td>
<td>231.7 ± 86.9</td>
<td>2.21 0.032*</td>
<td>0.011 0.92</td>
</tr>
<tr>
<td>HL (mm)</td>
<td>27.8 ± 5.5</td>
<td>45.7 ± 8.3</td>
<td>51.9 ± 8.2</td>
<td>2.77 0.008*</td>
<td>0.141 0.71</td>
</tr>
<tr>
<td>HW (mm)</td>
<td>17.0 ± 3.5</td>
<td>29.2 ± 5.9</td>
<td>33.0 ± 5.9</td>
<td>2.46 0.017*</td>
<td>0.008 0.93</td>
</tr>
<tr>
<td>HD (mm)</td>
<td>13.2 ± 2.7</td>
<td>23.6 ± 4.8</td>
<td>26.6 ± 5.0</td>
<td>2.33 0.024*</td>
<td>0.001 0.98</td>
</tr>
<tr>
<td>HC (mm)</td>
<td>54.4 ± 11.6</td>
<td>94.8 ± 18.2</td>
<td>106.1 ± 18.1</td>
<td>2.30 0.026*</td>
<td>0.010 0.92</td>
</tr>
<tr>
<td>UBL (mm)</td>
<td>9.6 ± 2.0</td>
<td>16.2 ± 3.2</td>
<td>18.3 ± 3.1</td>
<td>2.52 0.015*</td>
<td>1.78 0.19</td>
</tr>
<tr>
<td>UBD (mm)</td>
<td>13.6 ± 3.0</td>
<td>23.1 ± 4.0</td>
<td>26.5 ± 4.3</td>
<td>3.03 0.004*</td>
<td>0.462 0.50</td>
</tr>
<tr>
<td>UBW (mm)</td>
<td>5.7 ± 1.1</td>
<td>10.5 ± 2.0</td>
<td>12.1 ± 2.3</td>
<td>2.74 0.009*</td>
<td>0.44 0.51</td>
</tr>
<tr>
<td>LBL (mm)</td>
<td>9.0 ± 1.9</td>
<td>15.1 ± 3.1</td>
<td>17.0 ± 2.7</td>
<td>2.38 0.021*</td>
<td>0.14 0.71</td>
</tr>
<tr>
<td>LBW (mm)</td>
<td>10.7 ± 2.5</td>
<td>19.1 ± 3.6</td>
<td>21.5 ± 3.5</td>
<td>2.48 0.017*</td>
<td>1.77 0.19</td>
</tr>
<tr>
<td>LBD (mm)</td>
<td>4.4 ± 1.3</td>
<td>9.3 ± 2.8</td>
<td>11.1 ± 2.5</td>
<td>2.56 0.014*</td>
<td>1.73 0.19</td>
</tr>
<tr>
<td>SL (mm)</td>
<td>7.3 ± 2.1</td>
<td>15.0 ± 4.0</td>
<td>17.3 ± 3.5</td>
<td>2.36 0.023*</td>
<td>0.31 0.58</td>
</tr>
<tr>
<td>BF (N)</td>
<td>18.6 ± 9.1</td>
<td>77.2 ± 33.3</td>
<td>99.5 ± 32.7</td>
<td>2.45 0.018*</td>
<td></td>
</tr>
</tbody>
</table>

Significance levels \( (\alpha = 0.05) \). CL, carapace length; HL, head length; HW, head width; HD, head depth; HC, head circumference; UBL, upper beak length; UBW, upper beak width; UBD, upper beak depth; LBL, lower beak length; LBW, lower beak width; LBD, lower beak depth; SL, symphysis length; BF, bite force.
Table 1.2. Summary of reduced major axis regression analyses for ontogenetic scaling of craniofacial morphology and bite force in *Sternotherus minor*.

<table>
<thead>
<tr>
<th>Variables (y vs. x)</th>
<th>$R^2$</th>
<th>Intercept $(a)$</th>
<th>Slope $(b)$</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>$P$-value</th>
<th>Isometric Prediction</th>
<th>Growth Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL vs. CL</td>
<td>0.96</td>
<td>-0.49</td>
<td>1.07</td>
<td>1.01</td>
<td>1.13</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>HW vs. CL</td>
<td>0.97</td>
<td>-0.85</td>
<td>1.16</td>
<td>1.12</td>
<td>1.20</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>HD vs. CL</td>
<td>0.96</td>
<td>-1.03</td>
<td>1.20</td>
<td>1.14</td>
<td>1.26</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>HC vs. CL</td>
<td>0.97</td>
<td>-0.34</td>
<td>1.16</td>
<td>1.12</td>
<td>1.20</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>HW vs. HL</td>
<td>0.98</td>
<td>-0.32</td>
<td>1.08</td>
<td>1.04</td>
<td>1.12</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>HD vs. HL</td>
<td>0.96</td>
<td>-0.48</td>
<td>1.11</td>
<td>1.05</td>
<td>1.17</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>HC vs. HL</td>
<td>0.97</td>
<td>0.18</td>
<td>1.08</td>
<td>1.04</td>
<td>1.12</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>UBL vs. HL</td>
<td>0.96</td>
<td>-0.50</td>
<td>1.02</td>
<td>0.98</td>
<td>1.06</td>
<td>0.23</td>
<td>1</td>
<td>I</td>
</tr>
<tr>
<td>UBW vs. HL</td>
<td>0.97</td>
<td>-0.39</td>
<td>1.06</td>
<td>1.02</td>
<td>1.10</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>UBD vs. HL</td>
<td>0.95</td>
<td>-0.90</td>
<td>1.16</td>
<td>1.10</td>
<td>1.19</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>LBL vs. HL</td>
<td>0.97</td>
<td>-0.54</td>
<td>1.02</td>
<td>0.98</td>
<td>1.06</td>
<td>0.24</td>
<td>1</td>
<td>I</td>
</tr>
<tr>
<td>LBW vs. HL</td>
<td>0.98</td>
<td>-0.57</td>
<td>1.11</td>
<td>1.07</td>
<td>1.15</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>LBD vs. HL</td>
<td>0.97</td>
<td>-1.52</td>
<td>1.49</td>
<td>1.43</td>
<td>1.55</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>SL vs. HL</td>
<td>0.97</td>
<td>-1.11</td>
<td>1.37</td>
<td>1.31</td>
<td>1.43</td>
<td>&lt; 0.0001</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>BF vs. Mass</td>
<td>0.98</td>
<td>-0.32</td>
<td>0.98</td>
<td>0.94</td>
<td>1.02</td>
<td>&lt; 0.0001</td>
<td>0.67</td>
<td>P</td>
</tr>
<tr>
<td>BF vs. CL</td>
<td>0.97</td>
<td>-3.95</td>
<td>2.89</td>
<td>2.79</td>
<td>2.99</td>
<td>&lt; 0.0001</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>BF vs. HL</td>
<td>0.95</td>
<td>-2.47</td>
<td>2.60</td>
<td>2.46</td>
<td>2.74</td>
<td>&lt; 0.0001</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>BF vs. HW</td>
<td>0.96</td>
<td>-1.73</td>
<td>2.44</td>
<td>2.32</td>
<td>2.56</td>
<td>&lt; 0.0001</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>BF vs. HD</td>
<td>0.96</td>
<td>-1.35</td>
<td>2.34</td>
<td>2.22</td>
<td>2.46</td>
<td>&lt; 0.0001</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>BF vs. HC</td>
<td>0.97</td>
<td>-3.02</td>
<td>2.47</td>
<td>2.37</td>
<td>2.57</td>
<td>&lt; 0.0001</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>BF vs. UBL</td>
<td>0.93</td>
<td>-1.17</td>
<td>2.50</td>
<td>2.34</td>
<td>2.66</td>
<td>&lt; 0.0001</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>BF vs. UBW</td>
<td>0.96</td>
<td>-1.56</td>
<td>2.49</td>
<td>2.37</td>
<td>2.61</td>
<td>&lt; 0.0001</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>BF vs. UBD</td>
<td>0.94</td>
<td>-0.42</td>
<td>2.23</td>
<td>2.09</td>
<td>2.37</td>
<td>&lt; 0.0001</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>BF vs. LBL</td>
<td>0.94</td>
<td>-1.16</td>
<td>2.55</td>
<td>2.39</td>
<td>2.71</td>
<td>&lt; 0.0001</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>BF vs. LBW</td>
<td>0.97</td>
<td>-1.25</td>
<td>2.43</td>
<td>2.33</td>
<td>2.53</td>
<td>&lt; 0.0001</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>BF vs. LBD</td>
<td>0.96</td>
<td>0.15</td>
<td>1.76</td>
<td>1.66</td>
<td>1.86</td>
<td>&lt; 0.0001</td>
<td>2</td>
<td>N</td>
</tr>
<tr>
<td>BF vs. SL</td>
<td>0.95</td>
<td>-0.31</td>
<td>1.85</td>
<td>1.75</td>
<td>1.95</td>
<td>0.001</td>
<td>2</td>
<td>N</td>
</tr>
</tbody>
</table>

Significance levels ($\alpha = 0.05$). $P$-values were corrected using modified t-tests to reflect differences from isometric predictions. For growth types, $P =$ positive allometry, $I =$ isometry, and $N =$ negative allometry. CL, carapace length; HL, head length; HW, head width; HD, head depth; HC, head circumference; UBL, upper beak length; UBW, upper beak width; UBD, upper beak depth; LBL, lower beak length; LBW, lower beak width; LBD, lower beak depth; SL, symphysis length; BF, bite force.
Figure 1.3. Log-log plots for the scaling of head size measures on carapace length in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of each independent variable. (a) head length (HL; filled circles) and head width (HW; open circles), and (b) head circumference (HC; filled triangles) and head depth (HD; open triangles) as a function of carapace length (CL). P = positive allometry.
Figure 1.4. Log-log plots for the scaling of head measures on head length in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of each independent variable. Head width (HW; open circles), head depth (HD; open triangles), and head circumference (HC; filled triangles). P = positive allometry.
Figure 1.5. Log-log plots for the scaling of beak measures on head length in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of each independent variable. (a) upper beak width (UBW; filled circles), upper beak length (UBL; open circles), and upper beak depth (UBD; filled triangles), (b) lower beak width (LBW; filled circles), and lower beak length (LBL; open circles), and (c) symphysis length (SL; filled circles) and lower beak depth (LBD; open circles) as a function of head length (HL). P = positive allometry, and I = isometry.
Figure 1.6. Log-log plots for the scaling of bite force on body size in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of each independent variable. Bite force as a function of (a) carapace length (CL; filled circles) and (b) body mass (open circles). \( P = \) positive allometry.
Figure 1.7. Log-log plots for the scaling of bite force on head measures in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of each independent variable. Bite force as a function of head length (HL; filled circles), head width (HW; open circles), head depth (HD; open triangles), and head circumference (HC; filled triangles). P = positive allometry.
Figure 1.8. Log-log plots for the scaling of bite force on beak measures in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of each independent variable. Bite force as a function of (a) upper beak width (UBW; filled circles), upper beak length (UBL; open circles), and upper beak depth (UBD; filled triangles); (b) lower beak width (LBW; filled circles), and lower beak length (LBL; open circles); and (c) symphysis length (SL; filled circles) and lower beak depth (LBD; open circles). P = positive allometry, and N = negative allometry.
The ability of vertebrates to perform ecologically-relevant tasks is tightly linked to the morphology of the musculoskeletal system and ultimately determines patterns of resource (e.g., Kiltie 1982; Wainwright 1987; Hernández and Motta 1997; Husak et al. 2006; Bulté et al. 2008). Consequently, understanding how variation in musculoskeletal traits leads to differences in functional ability (i.e. performance) has become a central goal in fields of functional morphology and biomechanics (e.g., Arnold 1983; Wainwright 1987; Kingsolver and Huey 2003). In particular, the functional consequences of variation in cranial musculature and associated scaffolding on feeding performance have been studied across a broad range of taxa [elasmobranches (e.g., Wilga and Motta 2000; Huber et al. 2005, 2008; Lowry and Motta 2007), osteichthyans (e.g., Wainwright 1987, Hernández and Motta 1997; Huskins 1997; Hernández 2000; Westneat 2003, 2004; Herrel et al. 2005b; Westneat et al. 2005), lizards (e.g., Dalrymple 1977; Gans et al. 1985; Sinclair and Alexander 1987; Herrel et al. 1997, 1998, 1999, 2007; Moreno et al. 2008), turtles (e.g., Dalrymple 1977; Sinclair and Alexander 1987; this study), crocodilians (e.g., Cleuren and De Vree 1992; Cleuren et al. 1995; Daniel and McHenry 2000), birds (e.g. van der Meij and Bout 2004; Herrel et al. 2005a), and mammals (e.g., Kiltie 1982; Thompson et al. 2003; Huhov et al. 2005; Ellis et al. 2008; Herrel et al. 2008)]. In many of the aforementioned studies, biomechanical models of the feeding apparatus were developed in order to better understand the transmission of force and velocity within the musculoskeletal-feeding systems (Westneat 2004). Biomechanical models are valuable in such studies because they provide testable hypotheses of the relationship between morphology and performance (Westneat 2003, 2004), and allow investigators to determine which morphological traits control and
maintain the operation of musculoskeletal function (Scott and Winter 1991; Weishampel 1993). In addition, the identification of particular morphological traits important to ecological performance provide the impetus for exploring the mechanistic and functional issues that underlie the evolution of phenotypic traits (Arnold 1983; Kingsolver and Huey 2003).

Among performance traits relevant to vertebrate feeding, maximum bite-force generation has received considerable attention (see review in Anderson et al. 2008). Moreover, biomechanical models of maximum bite-force generation have been shown to be predictive when compared to empirically-measured bite forces (e.g., Sinclair and Alexander 1987; Herrel et al. 1999; Huber and Motta 2004; Huber et al. 2005; Herrel et al. 2008), electrically-stimulated bite forces (e.g., Huber and Motta 2004; Huber et al. 2005, 2008; Ellis et al. 2008), and prey-crushing forces (e.g., Kiltie 1982; Wainwright 1987; Hernández and Motta 1997). Particular interest has been given to organisms that require high bite forces to process and consume robust prey items (e.g., bone, mollusks, seeds), a foraging mode known as durophagy. Durophagy has been described in virtually all vertebrate groups [chondrichthyans (e.g., Summers 2000; Wilga and Motta 2000; Huber et al. 2005, 2008), osteichthyans (e.g., Wainwright 1987; Turingan and Wainwright 1993; Hernandez and Motta 1997; Grubich 2003, 2005), amphibians (Inger 1966), squamates (e.g., Dalrymple 1979a; Pregill 1984; Wineski and Gans 1984; Green et al. 1997; Jackson and Fritts 2004), turtles (e.g., Carr 1952; Legler and Georges 1993; Bonin et al. 2008), crocodilians (e.g., Dodson 1975; Cleuren and De Vree 2000; Brochu 2001), birds (e.g., van der Meij and Bout 2004; Herrel et al. 2005a), and mammals (e.g., Kiltie 1982; Werdelin 1989)]. Durophagy is almost invariably associated with morphological changes that allow consumers to expand their diet (e.g., Wainwright 1991; Binder and Van Valkenburgh 2000) or occupy niches for which there are few competitors (e.g., Wainwright 1987; Summers 2000; Herrel et al. 2002; Bulté et al. 2008). Because maximum bite-force generation is ecologically relevant to durophagy and is likely under strong selection for efficient design and performance, it is a useful parameter from which to examine the relationship between biomechanical design and performance.

An ontogenetic (or phylogenetic) increase in maximum bite-force generation is presumably required to inhabit a durophagous niche and can be achieved in several, often mutually non-exclusive, ways (Herrel et al. 2002). All vertebrates increase in size during development and changes in size are by necessity accompanied by increases in absolute muscle-
force generation (Hill 1950; Schmidt-Nielson 1984), therefore simply growing larger will inevitably lead to increases in absolute bite-force generation. As a result, maximum bite-force generation has been found to be strongly correlated with overall body size in all taxa studied (Anderson et al. 2008; Pfaller see Chapter 1). For the same reason, ontogenetic increases in the dimensions of the head (e.g., length, width, depth) in the absence of shifts in muscle physiology, lever mechanics, or body size, will also lead to an increase in maximum bite-force generation. All available data, however, from a broad range of taxa (see review in Herrel and Gibb 2006; Pfaller see Chapter 1) show that bite-force generation increases disproportionately relative to changes in head and body dimensions across ontogeny. Perhaps not surprisingly, these results suggest that the scaling of traits related to the musculoskeletal biomechanics of the feeding systems (e.g., lever mechanics, muscle mass, and/or muscle architecture) may have more explanatory power in terms of ontogenetic changes in bite-force generation than external head and body measures (Herrel et al. 2002; Pfaller see Chapter 1). Differential scaling of muscle mass and architecture, and lever mechanics, have been posited to explain the disproportionate increase in bite force, however relatively few detailed, quantitative studies have examined the musculoskeletal biomechanics of an ontogenetic series to support this observation. Furthermore, those that have are restricted to fishes (Wainwright 1987; Hernández and Motta 1997; Huber and Motta 2004; Grubich 2005; Herrel et al. 2005b; Huber et al. 2006, 2008).

In the absence of changes in size, biomechanical theory predicts that the force generating capacity of a musculoskeletal apparatus may be increased by improving the mechanical leverage of the system and/or by increasing muscle-force generation (Cochran 1982). Additionally, muscle-force generation is a function of the physiological cross-sectional area (PCSA) of the muscle fibers generating the force (Emerson and Bramble 1993), and can be increased by increasing the relative mass of the muscle or by changing the fiber architecture (i.e. the degree and angle of pennation, fiber length; Gans and De Vree 1987), or through a combination of the two.

The present study will test these hypotheses to explain the disproportionate increase in bite-force generation. Modifications to the lever mechanics of the feeding system may be the answer, if in-lever lengths increase allometrically relative to the out-lever length. Alternatively, increases in muscle mass alone may explain the disproportionate increase in bite-force generation, if changes in muscle mass scale with positive allometry relative to skull length such
that bite-force generation scales isometrically. Lastly, changes to the muscle fiber architecture (i.e. degree of pennation) may explain the disproportionate increase in bite-force generation, if the interaction between increasing pennation angles and decreasing fiber lengths results in bite-force generation scaling isometrically relative to $P_{\text{CSA}}$. Moreover, the disproportionate increase in bite-force generation may be achieved by a combination of one or more of these changes.

The edentulous, akinetic feeding apparatus of turtles provides a simple, yet relevant, system to conduct an investigation of the relationship between biomechanical morphology and performance. In addition, turtles represent an interesting group to study bite-force generation because the development of the feeding apparatus is believed to be constrained by the need to retract the head between the carapace and plastron (Dalrymple 1979b; Herrel et al. 2002). While considerable attention has been given to the organization of skull elements and musculature in turtles (e.g. Schumacher 1973; Gaffney 1979), limited data has been obtained on the biomechanical capacities of the jaws (e.g. bite force; except see Herrel et al. 2002; Herrel and O’Reilly 2006; Bulté et al. 2008; Pfaller see Chapter 1). In the present study, I will examined the relationship between bite-force generation and musculoskeletal feeding biomechanics in an ontogenetic series of loggerhead musk turtles, *Sternotherus minor*. It is well established that these turtles undergo dramatic morphological changes, in which adults develop hypertrophied skulls and beak surfaces (Carr 1952; Tinkle 1958; Pfaller see Chapter 1). These changes are posited to reflect a strong tendency towards increased durophagy across ontogeny (Ernst et al. 1994; Zappalorti and Iverson 2006), in which juveniles feed on a variety of prey items, such as insects, millipedes, spiders, crayfish, snails, clams, and fish (Tinkle 1958; Ashton and Ashton 1985), while adults primarily consume snails and clams (Carr 1952; Tinkle 1958). In addition, bite-force generation in *S. minor* has been shown to increase disproportionately relative to external morphological measures (Pfaller see Chapter 1), which suggests that changes to the musculoskeletal biomechanics of the feeding apparatus occur across ontogeny.

The overall goal of this work is to determine the scaling relationships between feeding biomechanics, bite-force generation, and increased durophagy in *S. minor*. To do this I will: (1) estimate bite-force generation based on biomechanical modeling of lever-arm mechanics and muscle-force generation from an ontogenetic series of *S. minor*, and compare these theoretical values with observed bite-force measurements obtained directly from the live individuals, (2) determine the scaling relationships among biomechanical variables, and between biomechanical
variables and bite-force generation, to explain the disproportionate increase in bite-force generation displayed by *S. minor* across ontogeny, and (3) place bite-force generation in the context of the dietary ontogeny of *S. minor* by estimating the force required to fracture snails found in their diet.

**Materials and Methods**

**Experimental Animals**

On 26 April 2008, an ontogenetic series composed of 30 *Sternotherus minor* [yearlings (26 g, 53.2 mm carapace length) to adults (331 g, 126.5 mm carapace length)] were hand captured while snorkeling in the Rainbow River, Marion Co., Florida, USA. Upon capture, specimens were held in shaded containers prior to data collection. At the field site, all individuals were measured for standard external morphometrics [carapace length and head length (= skull length); for descriptions see Pfaller Chapter 1] and subaerial bite-force generation. Animals were then transported to a nearby veterinary clinic and euthanized with an intravenous overdoes of pentobarbital sodium (390 mg/ml; 0.5 ml/individual) in accordance with the guidelines of the Institutional Animal Care and Use Committee of Florida State University (ACUC Protocol # 0011). Specimens were placed on ice and transported to the histology laboratory at Florida State University (Tallahassee, FL) to be dissected for biomechanical modeling of bite-force generation and dietary analysis.

**Observed Bite-Force Generation**

To measure bite-force generation from the ontogenetic series of 30 *S. minor*, I used the same protocol and apparatus as described by Pfaller (see Chapter 1). The apparatus was composed of a high impedance load cell (Kistler Instrument Corp., Type 9212, Amherst, NY) sandwiched between two stainless-steel cantilever beams, such that the voltage output routed through a charge amplifier (Kistler Instrument Corp., Type 5995A, Amherst, NY) was proportional to the compressive force applied to the pair of beams. Two bite-plate configurations were used depending on the size of turtle being tested (3 mm gape configuration for turtles less than 100 g; 5 mm gape configuration from turtles greater than 100 g). Animals
were restrained, and appropriate bite plates were placed unilaterally between the jaws, centered mesio-distally at the trough of the crushing surface along the lower beak. Each individual was measured for subaerial bite-force generation during a sequence of three bite-force trials separated by approximately two minutes. The highest of the three bite forces registered by an individual was treated in post hoc analyses as the maximum bite force.

**Morphology**

A theoretical model of bite-force generation in *Sternotherus minor* was designed by investigating the forces produced by the three muscles of the Musculus adductor mandibulae complex [(1) M. add. mand. externus, (2) M. add. mand. posterior, and (3) M. add. mand. internus; Figure 2.1], which collectively function to close (i.e. adduct) the jaws. The nomenclature for these muscles and their attachments follow Schumacher (1973): (1) The M. add. mand. externus is the largest jaw adductor in turtles and is superficially prominent. The bulk of this muscle is pennate with fibers inserting around a centrally located tendon (external tendon sensu Schumacher 1973) which attaches by a broad base to the bones of the coronoid process (dentary, coronoid, surangular, and prearticular). The external tendon rises posteromedially within the lower temporal fossa to the trochlear process (quadrate and prootic in cryptodirans) where it reflects via the cartilago transiliens (sliding cartilage) posteriorly within the upper temporal fossa towards the supraoccipital. Attached to the external tendon are the Pars superficialis laterally and the Pars profunda medially. The attachment area for the Pars profunda is enlarged by a secondary lobe of the external tendon running dorsally from the central tendon and an additional tendon running anteriorly from the supraoccipital bone (supraoccipital tendon sensu Schumacher 1973). As a result of this tendinous arrangement, the portion of the M. add. mand. externus that lies in the upper temporal fossa is multipennate and is composed of 4 muscle subdivisions [Pars superficialis and 3 bodies of the Pars profunda (α, β, γ); Figures 2.1a, b]. The Pars media is located entirely within the lower temporal fossa, but is not attached to the external tendon. The fibers of the Pars media originate from the anterior surface of the quadrate and run to the posterior edge of the coronoid process (surangular, coronoid, dentary; Figure 2.1b). (2) The M. add. mand. posterior is a small, parallel-fibered muscle located entirely within the lower temporal fossa. Its fibers originate on the rostral surface of the quadrate and prootic medially, and run ventrally converging on the medial side of the lower jaw (articular and prearticular;
Figure 2.1c). (3) The M. add. mand. internus lines the medial wall of the lower temporal fossa along with the M. add. mand. posterior, and together they form a muscular crescent (Schumacher 1973). The M. add. mand. internus can be further subdivided into the M. pseudotemporalis and M. pterygoideus. The fibers of the M. pseudotemporalis originate near the base of the orbit (parietal and prootic) and insert on the medial surface of the internal tendon, which attaches to the medial side of the lower jaw (prearticular and articular) near the jaw joint (Figure 2.1c). Similarly, the fibers of the M. pterygoideus originate from both the dorsal (descending process of the parietal and dorsal part of the palatine) and ventral (pterygoid, maxilla, quadrate) surfaces of the base of the orbit, and converge on the lamina anterior of the internal tendon (Figure 2.1c). In summation, the present study will consider eight muscle subdivisions that collectively function to adduct the lower jaw: Pars superficialis and Pars profunda α, β, γ (all attached to the external tendon), Pars media, M. posterior, M. pseudotemporalis, and M. pterygoideus.

Theoretical Bite-Force Generation

For each of the 30 specimens, all jaw adductor subdivisions were excised from the right side of the head, photographed (Nikon D40 digital camera, Nikon Corporation, Tokyo, Japan), and immediately weighed to the nearest thousandth of a gram (Ohaus® Adventurer™ Series Balance, Model AR-1530, Ohaus Corp., Pine Brook, NJ; Linearity = ± 0.002 g). Digital photographs were also taken of the head at multiple stages during each dissection from both lateral and dorsal views. For each subdivision, muscle fiber lengths (cm) and pennation angles (where applicable) were measured post-dissection from the aforementioned digital photographs (ImageJ v. 1.40, National Institute of Health, Bethesda, MD), and averaged from 10 random locations across the muscle body. Because the fiber lengths and pennation angles were variable among the muscle subdivisions attached to the external tendon (Pars superficialis and Pars profunda α, β, γ), these muscles were treated as four separate entities each generating its own force, and collectively acting to pull the external tendon and adduct the jaw (Figures 2.1a, b). Moreover, because the pennation in the M. add. mand. internus (M. pseudotemporalis and M. pterygoideus) is poorly developed, and particularly difficult to measure in small individuals, pennation angles for these muscles were not measured or considered in the theoretical bite-force estimates. Instead both the M. pseudotemporalis and M. pterygoideus were modeled as parallel-fibered muscles.
Using these data, the average physiological cross-sectional area (P_{CSA}; cm^2) of each muscle subdivision was computed as muscle mass (g) divided by average fiber length (cm) (Powell et al. 1984), assuming that muscle density is approximately 1 g cm^{-3} (McMahon 1984).

\[
P_{CSA} (cm^2) = \frac{\text{muscle mass (g)}}{\text{muscle density (g cm}^{-3})} \times \frac{1}{\text{fiber length (cm)}}
\]

The pennate arrangement of the fibers attached to external tendon (Pars superficialis and Pars profunda α, β, γ) required an additional term to account for the fact that pennate fibers attach obliquely to the tendon causing only a portion of the force generated by the muscle fibers to act in parallel to the tendon (Gans et al. 1985; Turawski et al. 1998). Consequently, the equations for the average P_{CSA} of the Pars superficialis and the three bodies of the Pars profunda (α, β, γ), include a term representing the cosine of the average pennation angle for each muscle body. This is represented in the following equation as θ (Powell et al. 1984; Lieber and Fridén 2000).

\[
P_{CSA} (cm^2) = \frac{\text{muscle mass (g)}}{\text{muscle density (g cm}^{-3})} \times \frac{1}{\text{fiber length (cm)}} \times \cos \theta
\]

The average P_{CSA} for each muscle subdivision was then multiplied by three different muscle stress values (20, 25, and 30 N cm^{-2}) to determine the theoretical maximum muscle-force (F_0) generated (Newtons):

\[
F_0 (N) = P_{CSA} (cm^2) \times \text{muscle stress (N cm}^{-2})
\]

Three different muscle stress values were used because actual muscle stress values for chelonian cranial muscle are not available. Empirically-determined values of maximum isometric muscle stress are between 10-40 N cm^{-2} for vertebrate skeletal muscle (Johnson and Gleeson 1984; Marsh 1988; Josephson 1993; Lou et al. 2002), and values of 20 N cm^{-2} (e.g., Kiltie 1984; Wainwright 1987; Hernández and Motta 1997; Huber and Motta 2004; Grubich 2005), 25 N cm^{-2} (e.g., Cleuren et al. 1995; Herrel et al. 1998, 1999, 2008), and 30 N cm^{-2} (e.g., Weijs and Hillen 1985; Sinclair and Alexander 1987; Thomason 1991; Daniel and McHenry 2000; Ellis et al. 2008) have been commonly used by investigators attempting to determine theoretical whole muscle-force generation in vertebrates.

Theoretical bite-force generation was then estimated by developing a static equilibrium model of the aforementioned forces generated by the jaw adductor muscles and the lever mechanics of the feeding system (e.g., Cleuren et al. 1995; Herrel et al. 1998, 2008; Huber et al. 2005, 2008). Using the tip of the snout as the origin of a three-dimensional coordinate system,
the origins and insertions of each muscle, as well as the positions of the jaw joint (quadrate/articular) and bite point, were determined by measuring the distances of these points from the origin (Figure 2.2). Digital photographs that were made when specimens were dissected and were used to develop the three-dimensional coordinate systems for each individual. For all individuals, the orientation of muscles was determined for one specific state of jaw depression: a gape angle of approximately 10°. This angle represents an approximately closed gape similar to when prey items are being crushed. Force vectors were created for each muscle based on their theoretical maximum muscle force \( F_0 \) and three-dimensional coordinates of the origin and insertion. Because the model used to calculate the static equilibrium was planar (two-dimensional), only the sagittal component of each force vector was considered in the model (i.e. the medial Cartesian vector was removed). This assumption is does not compromise the model because the major adductor muscles in reptiles do not generally have large angles in the medial plane (Sinclair and Alexander 1987). Furthermore, when biting activity is symmetrical as in this system, such lateral and medial forces are predominantly canceled out (Cleuren et al. 1985).

Additionally, in-lever lengths for each muscle \( IL \) were measured as the perpendicular distance from the three-dimensional coordinates of the jaw joint to the muscle-force vector (Figures 2.2b-e). The Pars superficialis and Pars profunda (\( \alpha, \beta, \gamma \)) attach to the external tendon and act along the same lever length (Figures 2.2a, b). The out-lever length for biting \( OL \) at the trough of the crushing surfaces along the lower jaw (directly lateral to the mid-jaw symphysis; Figure 2.2) is the same for all muscles within a given specimen and was determined from the three-dimensional coordinates of the bite point and the jaw joint. For a jaw system in static equilibrium, the rotational forces (i.e. moments) generated by the muscular forces acting about the jaw joint (fulcrum) must balance the force of biting (Cochran 1982; Witmer and Rose 1991; Ellis et al. 2008). Maximum bite-force generation \( BF \), therefore, was estimated by the following equation:

\[
BF = \frac{(F_{sup,pro} \times IL_{sup,pro})}{OL} + \frac{(F_{med} \times IL_{med})}{OL} + \frac{(F_{post} \times IL_{post})}{OL} + \frac{(F_{pseudo,ptery} \times IL_{pseudo,ptery})}{OL},
\]

where \( F_{sup,pro} \) and \( IL_{sup,pro} \) are the muscle force and in-lever length, respectively, for the Pars superficialis and Pars profunda collectively (Figure 2.2b); \( F_{med} \) and \( IL_{med} \) are the muscle force and in-lever length for the Pars media, respectively (Figure 2.2c); \( F_{post} \) and \( IL_{post} \) are the muscle
force and in-lever length for the M. posterior, respectively (Figure 2.2d); $F_{\text{pseudo,ptery}}$ and $IL_{\text{pseudo,ptery}}$ are the muscle force and in-lever length, respectively, for the M. pseudotemporalis and M. pterygoideus collectively (Figure 2.2e); and OL is the out-lever length measured from the jaw joint to the bite point (Figures 2.2 b-e). The forces acting at the jaw joints (i.e. joint reaction forces) are important for maintaining the static equilibrium of the jaw mechanism by balancing the forces acting on the jaws (e.g., Sinclair and Alexander 1987; Huber et al. 2008), however in the present study the goal was to evaluate the mechanical forces in terms of purely rotational and translational components relative to the jaw joint (Weishampel 1993). As a result, joint reaction forces were considered, and they are not discussed further in terms of theoretical bite-force generation.

Lastly, to account for the left-side (or balancing-side) musculature that was not excised during dissection, I multiplied BF by 2.0 to estimate the total bite-force generation (TBF). Some theoretical bite-force models assume simultaneous and maximum (i.e. 100%) contraction of the adductor musculature during biting (e.g., Wainwright 1987; Thomason 1991; Hernández and Motta 1997; Grubich 2005). Electromyographical work on reptiles, however, suggests that the degree of contraction may be variable due to variation in recruitment levels (Smith 1982; De Vree and Gans 1984; Gans et al. 1985; Cleuren et al. 1995). To explore the ramifications of such variation, TBF was then multiplied by 1.0, 0.9, or 0.8 to model 100% (maximal), 90%, and 80% contraction, respectively.

**Force to Fracture Experiments**

The force required to fracture (force to fracture = FF) snails [*Goniobasis (= Elimia)*] commonly consumed by *Sternotherus minor* (e.g., Tinkle 1958; Berry 1975) was determined through a series of mechanical loading experiments. To provide ecologically relevant data on dietary items, the effects of scaling on snail size was examined. Specifically, the sizes of snails consumed by each individual were determined by recovering opercula from the digestive tracts. Digestive tracts (esophagus to rectum) were excised and contents were removed. Volumetric data for the contributions of dietary items were not documented in the present study because items in the digestive tract are at variable stages of digestion and results would be skewed by more durable prey (i.e. snail shells). All detectable opercula were recovered and measured for maximum length (OL) from digital photographs (ImageJ v. 1.40, National Institute of Health,
Snail sizes were reconstructed based on the relationship between OL and maximum snail length (SnL) from a sample of 50 wild caught snails. Operculum length was a strong predictor of SnL ($R^2 = 0.94$, $t_{48} = 26.3$, $P < 0.0001$: $\text{SnL} = 1.55 + 3.83\times\text{OL}$; Figure 2.3), therefore it was used as a proxy for estimating snail size.

Using a mechanical loading frame (Model 312.31, Materials Testing Solutions, Eden Prairie, MN), the FF for *Goniobasis* was tested within the jaws of three different sized turtles (large - 306 g; medium – 174 g; small – 85 g). Two different load cells were used in the mechanical loading frame: 250 N (Model 41-0571-04-01, Sensatec® RF Technologies, Brookfield, WI) for the medium and small turtles, and 5000 N (Model 661.19e-01, Materials Testing Solutions, Eden Prairie, MN) for the large turtle. For each turtle, a size series of *Goniobasis* representing the range of snails consumed by turtles of comparable size were tested. Turtle heads were aligned upside down in a custom-built frame such that the head was braced laterally and the jaw joint was restrained to resist ventral disarticulation. Live *Goniobasis* were aligned between the jaws in a consistent orientation: the widest whorl contacting the flat crushing surfaces of the upper and lower beak. This location represents that site along the jaw that *S. minor* processes snails (Pfaller personal observation), as well as the location that was used to test and model bite-force generation (see above; Pfaller see Chapter 1). During a typical loading trial, compressive force was applied at a constant rate (0.5 mm s$^{-1}$) to the ventral limit of the mid-jaw symphysis until fracture of the snail was achieved. Because snail shell fragments found in the digestive tract do not show signs of catastrophic obliteration, loading trials were terminated when fracture of the first whorl was observed. This method is consistent with observations of feeding behavior, in which live individuals typically fracture only the first (and occasionally the second) whorl prior to ingestion (Pfaller personal observation).

**Statistical Analysis**

Except for descriptive statistics, all data were logarithmically transformed for statistical analyses. Observed bite-force generation measured from live individuals and theoretical estimates of bite-force generation determined from musculoskeletal dissections were regressed (reduced major axis regression) against skull length (standard index of head size; Bever 2008). Modified t-tests were used to test for statistically supported differences in slope. The relationship between theoretical and observed bite-force generation was determined by

Scaling relationships among morphological variables, and between morphological variables and bite-force generation (observed), were determined from reduced major axis regressions and compared with predictions based on isometric scaling (= “geometric similarity”; Schmidt-Nielson 1984). A simple model of isometric scaling was used as a null hypothesis against which empirically determined coefficients of scaling were compared (Hill 1950; Emerson and Bramble 1993). The isometric scaling model is based on Euclidean geometry and predicts that as an object grows in linear dimensions by \( n \), its area measures increase by \( n^2 \) and volume (or mass) by \( n^3 \) (Hill 1950; Schmidt-Nielson 1984; Emerson and Bramble 1993). Regression coefficients for all isometric scaling predictions are shown in Table 2.1. Reduced major axis (RMA) regressions on log-transformed data yielded equations in the form:

\[
\log y = \log a + b \log x,
\]

in which \( x \) is the independent variable, \( y \) is the dependent variable, \( a \) is the \( y \) intercept, and \( b \) is the scaling or regression coefficient (Sokal and Rohlf 2000). The scaling coefficient describes the relative allometry or isometry of the relationship. Deviations from isometry (i.e., allometry) were considered significant if the predicted slopes fell outside of the 95% confidence intervals of the observed slopes. Scaling coefficients significantly greater or less than those predicted by isometry were designated as positive or negative allometry, respectively. Reduced major axis regressions were plotted with the appropriate isometric relationship superimposed on each plot.

All reduced major axis regressions were done using RMA for Java v. 1.21 (Bohonak and van der Linde 2004).

To evaluate the dietary ontogeny of snail consumption, the number of snails (= opercula) and estimated size of snails found in the diet were regressed against skull length. The regression equation (RMA; Figure 2.14a) of FF on snail size was used to extrapolate the force necessary to fracture snails of estimated dimensions (based on operculum length) found in the digestive tracts of turtles. For each turtle, the FF of the five largest snails found in the diet were averaged (\( A_{FF} \)) and compared with bite-force generation (observed and maximum theoretical) using paired t-tests. Regression slopes (RMA) of the \( A_{FF} \) and bite force [observed and maximum theoretical (100% recruitment and 30 N cm\(^{-2}\) muscle stress)] scaled to skull length were compared using modified t-tests (R for Windows v. 2.8.1, The R Foundation for Statistical Computing, 2008).
Results

Morphology and Theoretical Bite-Force Generation

The M. adductor mandibulae externus, which is the primary jaw adductor in turtles (Schumacher 1973), was the largest of all muscles in *Sternotherus minor* (~ 91% of total muscle mass), and contributed the greatest amount to the total physiological cross-sectional area (P_{CSA}; ~ 86%) and bite-force generation (~ 98%; Table 2.2). Of the muscle subdivisions within the M. add. mand. externus, the Pars profunda α was the largest (~ 45 %), and contributed the greatest amount to total P_{CSA} (~ 32 %) and bite-force generation (~ 36%; Table 2.2). The M. add. mand. posterior and internus were particularly small (1.6 and ~ 7.5 % of total muscle mass, respectively), and contributed very little functional force to bite-force generation (0.6 and ~ 1.4 %, respectively; Table 4). Pertinent data not displayed in Table 2.2 are descriptive statistics for skull length (3.66 cm ± 0.19, 2.22 – 5.52 cm), observed bite-force generation (55.0 N ± 7.28, 7.09 – 124.6 N) and out-lever length (1.73 cm ± 0.11, 0.96 – 2.68 cm) for the 30 specimens used in this study.

Regression coefficients (i.e. slopes) for observed and theoretical bite-force generation scaled to skull length (SL) were significantly different from the isometric scaling prediction of 2.0 (Table 2.3) and were not statistically different from each other (observed: slope = 2.67, 95% CI = 2.50 – 2.84; theoretical: slope = 2.71, 95% CI = 2.54 – 2.88; Figure 2.4 and Table 2.3). Moreover, the slope for the regression between theoretical and observed bite-force generation was not significantly different from 1.0 (b = 1.03, t_{28} = 0.94, P = 0.18). Regression slopes for all nine theoretical bite-force estimates are identical because each represents P_{CSA} multiplied by a different combination of numbers, in which only the absolute values are changing. For the nine theoretical bite-force estimates representing three different muscle stress values (20, 25, and 30 N cm\(^{-2}\)) and three different percentages of total muscle recruitment (80, 90, and 100%), two were not statistically different from observed bite-force generation (80% contraction at 30 N cm\(^{-2}\) and 90% contraction at 25 N cm\(^{-2}\); Figure 2.4 and Table 2.4). Seven theoretical bite-force models were statistically different from observed bite-force generation: four were significantly less (all at 20 N cm\(^{-2}\) and 80 % contraction at 25 N cm\(^{-2}\)) and three were significantly greater (90%
contraction at 30 N cm$^{-2}$, 100% contraction at 25 N cm$^{-2}$, and 100% contraction at 30 N cm$^{-2}$; Figure 2.4 and Table 2.4).

Scaling of Feeding Biomechanics

The in-lever length (IL) of the Pars superficialis, profunda (IL$_{sup,pro}$) complex scaled isometrically ($b = 1.0$) relative to out-lever length (OL), and the in-lever lengths of the Pars media (IL$_{med}$), M. posterior (IL$_{pos}$), and M. pseudotemporalis/pterygoideus (IL$_{psedu,ptery}$) scaled with negative allometry ($b < 1.0$) relative to OL (Table 2.5 and Figure 2.5). As a result, the relative amount of muscle force being transferred to bite force was constant across ontogeny for the Pars superficialis, profunda complex, while the magnitude of the muscle force realized as bite force decreased across ontogeny for the Pars media, M. posterior, and M. pseudotemporalis, pterygoideus complex.

Changes in muscle mass of isolated muscle subdivisions mostly scaled with positive allometry ($b > 3.0$) relative to skull length (SL: Table 2.6a and Figures 2.6a-e, g). The two exceptions were the M. posterior and M. pterygoideus which scaled isometrically ($b = 3.0$ and 2.0, respectively; Table 2.6a and Figures 2.6f, h). In addition, total muscle mass scaled with positive allometry relative to SL ($b > 3.0$; Table 2.6a and Figure 2.7). Observed bite-force generation, however, scaled with positive allometry ($b > 0.67$) relative to the masses of each isolated muscle subdivision (Table 2.6b and Figure 2.8) and to the total muscle mass (Table 2.6b and Figure 2.9). Consequently, there is a disproportionate increase in the size of the adductor musculature across ontogeny in S. minor, however such changes do not scale in proportion with changes in bite-force generation.

Fiber lengths and fiber angles of the Pars superficialis and Pars profunda $\alpha$, $\beta$, $\gamma$ mostly scaled with negative allometry ($b < 0.34$) and positive allometry ($b > 0$), respectively, relative to their respective muscle masses (Table 2.7a, b and Figures 2.10a-b, d-h). Moreover, fiber lengths of the M. posterior, M. pseudotemporalis, and M. pterygoideus scaled isometrically relative to their respective muscle masses ($b = 0.34$; Table 2.7a) and the fiber lengths of the Pars media scaled with negative allometry relative to muscle mass ($b < 0.34$, Table 2.7b). Bite-force generation mostly scaled isometrically ($b = 1.0$) relative to the P$_{CSA}$ of each isolated muscle subdivision (Table 2.7c and Figures 2.6a-e, g), with the exceptions being the Pars profunda $\alpha$, M.
posterior, and M. pterygoideus (Table 2.7c and Figures 2.11a, f, h). Bite-force generation scaled isometrically relative to total P_{CSA} of the M. add. mand. (Table 2.7c and Figure 2.12).

**Force to Fracture Experiments**

Three-hundred and eighty-two opercula were recovered from the digestive tracts of the 30 turtle specimens (range: 5 – 52 per specimen). The number of opercula per specimen did not differ across the ontogenetic series (RMA on SL: R^2 = 0.0526, t_{28} = 1.25, P = 0.223; Figure 2.13a), however there was a highly correlated, positive exponential relationship between average snail size (estimated from opercular length: SnL = 1.55 + 3.83*OL; Figure 2.3) found in the diet and skull length (R^2 = 0.89, y = 2.797e^{0.0357x}; Figure 2.13b).

One-hundred and thirteen snails were tested for force to fracture (FF) in the mechanical loading frame [large turtle (n = 58: range 15.03 – 26.98 mm), medium turtle (n = 34: range 4.85 – 18.02 mm), and small (n = 21: range 4.52 – 10.15 mm)]. Of these 113 loading trials, 33 resulted in snails failing catastrophically (up to 676 N). In three others the snail slipped from the beak during loading prior to failure. These trials were not included in the post hoc analyses. Seventy-seven trials (large turtle, n = 30; medium turtle, n = 29; small turtle, n = 18) resulted in the snail shell fracturing in the characteristic pattern described above, in which only the first (or second) whorl is fractured. Collectively, there was a significant positive relationship between the FF and snail length (SnL) (RMA: R^2 = 0.76, t_{75} = 19.32, P < 0.0001: FF = 13.106*SnL – 60.09; Figure 2.14a). In addition, FF scaled isometrically relative to SnL (RMA on log-log data: regression slope = 1.77, R^2 = 0.72, t_{75} = 1.53, P = 0.07; Figure 2.14b). Using the reduced major axis regression equation from the untransformed data, I extrapolated the force required to fracture snails of estimated dimensions (based on operculum length) found in the digestive tracts of turtles. The average estimated FF of the five largest snails found in the diet of each turtle (A_{FF}) was significantly greater than the maximum theoretical bite-force estimations (100% recruitment and 30 N cm^{-2} muscle stress: t_{29} = 5.11, P < 0.0001) and the observed bite-force generation measured from live individuals (t_{29} = 9.50, P < 0.0001). There was a significant positive relationship between A_{FF} and skull length across ontogeny (R^2 = 0.88, t_{28} = 14.76, P < 0.0001; Figure 2.15a). The regression slope of A_{FF} scaled to skull length was significantly greater than the isometric scaling prediction of 2.0 (t_{29} = 2.81, P = 0.0044; Figure 2.15a), and was not statistically different from the regression slopes of theoretical bite-force generation (t_{29} =
1.437, \( P = 0.081 \) or observed bite-force generation (\( t_{29} = 1.197, P = 0.121 \)) scaled to skull length (Figure 2.15b).

**Discussion**

There are four major findings of this study. First, bite forces in *Sternotherus minor* are almost entirely generated by the M. adductor mandibulae externus. The bulk of this muscle is multipennate, which dramatically elevates the total physiological cross-sectional area of the adductor musculature and the capacity for bite-force generation. Second, the theoretical bite-force model developed in this study accurately predicts individual bite forces, and the ontogenetic scaling of bite-force generation, in *Sternotherus minor*. This demonstrates that the underlying morphology of the musculoskeletal system is a good predictor of observed performance across ontogeny. As a result, the model can be used as a means to test hypotheses regarding how *S. minor* (and other durophagous vertebrates) achieve disproportionately high bite-force generation across ontogeny. Third, bite-force generation in *S. minor* scaled with positive allometry relative to total muscle mass and isometrically relative to total muscle physiological cross-sectional area. These results suggest that changes in muscle architecture (fiber lengths and degrees of pennation) are important for *S. minor* to achieve disproportionately high bite-force generation across ontogeny, while retaining a head that is small enough to be retracted inside the shell. Finally, the forces required to fracture the largest *Goniobasis* snails found in the diet of *S. minor* scale with the same allometric increase as bite-force generation. This result indicates that the capacity of these animals to generate bite force is directly related to the fracture strength of their snail prey. Consequently, it is the ability to process disproportionately more robust prey that drives selection for a disproportionate increase in bite-force generation in *S. minor*.

**Morphology**

In *S. minor*, the M. add. mand. externus is by far the largest by mass of all eight jaw adductor subdivisions examined in this study. Within this muscle, the multipennate arrangement
of the Pars superficialis and Pars profunda dramatically elevates the total physiological cross-sectional area of the adductor musculature and the capacity for bite-force generation. Lastly, the attachment site and orientation of the external tendon, and its association with the trochlear process, provides these muscle bodies with elevated mechanical leverage required for high force transmission. For these reasons, the M. add. mand. externus generates 98% of the functional bite force in these animals. The dominance of the M. add. mand. externus in turtles is well established (Schumacher 1973; Dalrymple 1979b; Sinclair and Alexander 1987) and multipennate M. add. mand. externus have been described in other, forceful-biting turtles (trionychids and Podocnemis; Schumacher 1973). This muscle accounts for approximately 73% of the adductor muscle mass in Apalone (=Trionyx) ferox (Dalrymple 1979b). The M. add. mand. externus in Chrysemys picta represents 88% of the adductor muscle mass, but apparently generates only 75% of the bite force (Sinclair and Alexander 1987). Interestingly, in C. picta the M. add. mand. externus is not multipennate as in S. minor, and generates proportionally less bite force.

Such morphologies are hypothesized as being related to the fact that turtles are constrained by the need to retract their heads between the margins of their shell (Dalrymple 1979b; Herrel et al. 2002), as an antipredation tactic. Assuming that bite-force generation is ecologically relevant, the trochlear process and associated intramuscular cartilage at the junction of the upper and lower temporal fossae allows turtles to place adductor masses at the posterior half of the skull without losing substantially in mechanical leverage (Dalrymple 1979b; see Figure 2.2). The evolution of this muscular arrangement likely facilitated the enlargement of the M. add. mand. externus in the upper temporal fossa without substantially increasing the width and depth of the head, thus retaining the ability to retract the head inside the shell. Furthermore, the development of the multipennate muscle-fiber arrangement from the more common bipennate pattern seen in most turtles, likely allows durophagous turtles to increase the force-generating capacity of their adductor muscles without dramatically increasing the muscle size. Additional support for the presence of this limitation comes from the fact that S. minor have a well-developed transverse hinge between the epiplastron and hyoplastron bones that allow the plastron to be depressed to accommodate an enlarged head (Bramble et al. 1984)

For S. minor, however, this morphology does not come without a cost. Biomechanical theory predicts that a musculoskeletal apparatus that is proficient at generating high forces will
sacrifice in the ability to generate speed at multiple levels (Cochran 1982). Lever systems with high force transmission (i.e. high mechanical leverage), like that of *S. minor*, are typically maximized with relatively short out-levers, whereas speed is optimized through relatively long out-levers (i.e. low mechanical leverage) (Herrel *et al.* 2002). At the muscular level, pennate-fiber arrangements with high $P_{\text{CSA}}$ are designed for forceful, sustained contractions, whereas parallel-fiber arrangements are capable of short, fast bursts of activity over the greatest range of contraction (Cochran 1982; Gans *et al.* 1985; Lieber and Fridén 2000). For these reasons, it seems that the evolution of high bite-force generation required for durophagy in *S. minor* has come at the expense of jaw closing speed, and therefore the ability to feed on fast, elusive prey (Herrel *et al.* 2002). Particularly as adults, fast jaw closing behaviors involved in suction feeding are poorly developed (Pfaller personal observation), and agile prey (i.e. fish) are rarely found in the diet (Tinkle 1958). *Sternotherus minor* also has a relatively large tongue and weakly developed hyoid apparatus characteristic of more terrestrial turtles (Bramble 1973). There is an inverse relationship between the relative development of the hyoid apparatus and the tongue that is typically associated with terrestrial inertial feeding (large tongue, small hyoid) and aquatic suction feeding (small tongue, large hyoid) (Bramble 1973; Bramble and Wake 1985). Consequently, for *S. minor* the development of a mobile, muscular tongue, perhaps used to orally manipulate snails, may have come at a cost to the development of the hyoid apparatus and the ability to suction feed effectively. Further comparative studies between piscivorous and durophagous turtles would be particularly enlightening with regard to the functional tradeoffs between generating force and speed in musculoskeletal feeding systems.

**Theoretical Bite-Force Generation**

Adult *S. minor* are theoretically capable of generating bite forces of up to 172 N (100% recruitment, 30 N cm$^{-2}$). Although maximum theoretical estimates (30 N cm$^{-2}$ and 100% recruitment) typically overestimate the observed values, the theoretical bite-force model developed in this study accurately predicts the scaling of bite-force generation across ontogeny. When scaled to skull length, scaling coefficients for observed ($b = 2.67$) and theoretical ($b = 2.71$) bite-force generation were not significantly different. Furthermore, the scaling relationship between bite-force generation and head length ($b = 2.60$) in a different ontogenetic series of *S. minor* was not significantly different from the scaling coefficients in the present study ($n = 75$;
That the model accurately predicts the scaling of bite-force generation suggests that the underlying morphology of the musculoskeletal system (i.e. lever lengths, muscle masses, fiber lengths, and pennation angles) is a good predictor of observed performance, and that the biomechanical theory used to develop the model is sound.

Similar biomechanical models of bite-force generation across truncated growth series have been shown to be predictive when compared to empirically-measured bite forces (e.g., Sinclair and Alexander 1987; Herrel et al. 1999, 2008; Huber and Motta 2004; Huber et al. 2005), electrically-stimulated bite forces (e.g., Huber and Motta 2004; Huber et al. 2005; Ellis et al. 2008), and prey-crushing forces (e.g., Kiltie 1982; Grubich 2005) across a broad range of taxa. Attempts to model bite-force generation across ontogeny, however, are few, and those that have are restricted to fishes (Wainwright 1987; Hernández and Motta 1997; Huber and Motta 2004; Grubich 2005; Herrel et al. 2005b; Huber et al. 2006, 2008). Studies by Wainwright (1987) and Hernández and Motta (1997) compared theoretical bite force with prey-crushing forces in ontogenetic series of teleost fishes (*Lachnolaimus maximus* and *Archosargus probatocephalus*, respectively) and found that these forces increase at the same rate. In addition, Huber and colleagues (2008) compared theoretical bite-force generation with electrically-stimulated bite forces in an ontogenetic series of chimaera (*Hydrolagus colliei*), and found that the regression slopes of electronically-stimulated and theoretical bite force were not statistically different. Given that bite-force generation has been shown to be an important determinant of resource use across ontogeny (Hernández and Motta 1997), it is surprising that so few studies have attempted to develop biomechanical models that would allow investigators to determine which morphological traits control and maintain the operation of this musculoskeletal function.

To model bite-force generation, investigators typically assume one of three muscle stress values: 20 N cm\(^{-2}\) (e.g., Kiltie 1982; Wainwright 1987; Hernández and Motta 1997; Huber and Motta 2004; Grubich 2005), 25 N cm\(^{-2}\) (e.g., Cleuren et al. 1995; Herrel et al. 1998, 1999, 2008), and 30 N cm\(^{-2}\) (e.g., Weijs and Hillen 1985; Sinclair and Alexander 1987; Thomason 1991; Daniel and McHenry 2000; Ellis et al. 2008). Empirically-determined values of maximum isometric muscle stress are between 10-40 N cm\(^{-2}\) for vertebrate skeletal muscle (Johnston and Gleeson 1984; Marsh 1988; Josephson 1993; Lou et al. 2002), so these values are well within the possible range. Because muscle stress values for chelonia cranial muscles are unknown, I modeled the three common values with the goal of identifying which value best fit the observed
data. In addition, because electromyographical work in reptilian systems suggests that the level of contraction may be variable due to variation in recruitment levels (Smith 1982; Gans et al. 1985; De Vree and Gans 1984; Cleuren et al. 1995), I also modeled theoretical bite forces at 100%, 90%, and 80% recruitment. Once again these values were modeled with the goal of identifying which value best fit the observed data.

The results of bite-force modeling the nine different combinations of muscle stress and muscle recruitment for the adductor muscles of *S. minor* provide some insights into possible variation in muscle-force generation. First, it can be concluded that muscle stress values are probably greater than 20 N cm$^{-2}$. Muscle stress values below 20 N cm$^{-2}$ are unlikely because even at 100% recruitment observed bite-force generation was greater than the theoretical estimates. This conclusion is not surprising as 20 N cm$^{-2}$ is a value typical of fish white muscle (Josephson 1993), and chelonian cranial muscles are likely highly oxidative (i.e. red; Pfaller personal observation). Second, muscle stress is likely between 24 N cm$^{-2}$ and 30 N cm$^{-2}$. The observed data fits best at values between just below 25 N cm$^{-2}$ and 30 N cm$^{-2}$, which is consistent with muscle stress values typically used in muscle-force estimation for reptiles (e.g., Cleuren et al. 1995; Herrel et al. 1998, 1999; Sinclair and Alexander 1987; Daniel and McHenry 2000) and mammals (e.g., Weijs and Hillen 1985; Thomason 1991; Ellis et al. 2008; Herrel et al. 2008). Finally, muscle recruitment may be sub-maximal because at muscle stress values of 25 N cm$^{-2}$ and 30 N cm$^{-2}$ the observed bite-force measurements best fit the data modeling 90% and 80% recruitment, respectively. These results are consistent with the concerns that many muscle-modeling studies are often based on two questionable assumptions: all muscles act maximally and simultaneously (De Vree and Gans 1984; Cleuren et al. 1995). In this case, sub-maximal muscle recruitment best fits the observed data at typical muscle stress values and suggests that either muscle recruitment is typically sub-maximal or that individuals were not biting the transducer at or near maximum force (Anderson et al. 2008). The latter finds poor support in that the observed data and the estimated values are in accordance.

**Scaling of Feeding Biomechanics**

Bite-force generation (observed and theoretical) in *S. minor* scales with significant positive allometry relative to skull length. All previous studies across a broad range of vertebrate taxa, including *S. minor* (Pfaller see Chapter 1), show a similar pattern of significant positive
allometry of bite force relative to body and head dimensions (see review in Herrel and Gibb 2006; Huber et al. 2006, 2008). The consistency of this conclusion provided the impetus for exploring the scaling of musculoskeletal biomechanics across ontogeny to explain how these animals increase bite-force generation disproportionally during growth.

In the absence of changes in size, biomechanical theory predicts that the force-generating capacity of a musculoskeletal apparatus may be increased by improving the mechanical leverage of the system and/or by increasing muscle-force generation (Herzog 2007). The in-lever of the largest, most forceful jaw adductor muscles in *S. minor*, the Pars superficialis and Pars profunda, increased proportionately to changes in the out-lever (i.e. bite lever). Consequently, the mechanical leverage of these muscles is constant across ontogeny and do not contribute to relative increases in bite-force generation. Additionally, the in-levers of the smaller, less forceful jaw adductor muscles, the Pars media, M. posterior, and M. internus (pseudotemporalis and pterygoideus), tend to lose mechanical leverage by lengthening at a slower rate than the out-lever. These results indicate that changes to the mechanical leverage of the musculoskeletal feeding apparatus were not responsible for the disproportionate increase in bite-force generation across ontogeny in *S. minor*.

As previously mentioned, muscle-force generation can be increased by increasing the relative mass of the muscle or by changing the fiber architecture (i.e. the degree and angle of pennation, fiber length; Gans and De Vree 1987), or through a combination of the two. The total muscle mass of the M. adductor mandibulae increased with strong positive allometry relative to skull length. The muscle masses of the Pars superficialis and Pars profunda had the highest scaling coefficients of the eight muscle subdivisions. The muscle masses of the M. posterior and M. pterygoideus were particularly small and had scaling coefficients that did not deviate from isometry. This suggests that generating bite forces may be their subordinate role. The hypertrophy of the M. add. mand. externus and M. pseudotemporalis, however, do not account for all ontogenetic changes in bite-force generation: bite-force generation scales with positive allometry relative to muscle mass (total and isolated). These results indicate that changes in muscle mass are rather dramatic during ontogenetic growth in *S. minor*, however these changes only partially explain the disproportionate increase in bite-force generation.

The Pars superficialis, profunda complex in *S. minor* has a multipennate arrangement and generates a large percentage of the functional bite force. For a given muscle mass, as pennation
angles increase fiber lengths decrease (Huijing and Woittiez 1984; Muhl 1982; Woittiez et al. 1984), which ultimately leads to an increase in P_{CSA}. In addition, muscle hypertrophy has been shown to increase muscle-fiber pennation angles (Kawakami et al. 1993). These phenomena are what occur in S. minor across ontogeny. In general, pennation angles increase and fiber lengths decrease relative to changes in muscle mass, resulting in an ontogenetic increase in P_{CSA} of Pars superficialis and Pars profunda muscles. In addition, muscle fibers of the Pars media become relatively shorter, while muscle fibers of the M. posterior, M. pseudotemporalis, and M. pterygoideus scale isometrically. Consequently, the combined effects of increasing muscle masses and changing the muscle architecture of the pennate muscle bodies results in an increase in total P_{CSA} of the jaw adductor muscles that is proportional to changes in bite-force generation. These results indicate that the disproportionate increase in bite-force generation relative to skull length found in S. minor, is explained by allometric changes in muscle size and architecture that collectively act to elevate the P_{CSA} and muscle force. Lastly, these results suggest that changes in muscle architecture are important for increasing bite-force generation while maintaining relative muscle and head size, thus supporting the premise that turtle head size may be constrained by the need to retract the head between the margins of the shell (Dalrymple 1979b; Herrel et al. 2002).

Although ontogenetic studies on scaling of functionally relevant biomechanical properties, such as P_{CSA}, lever lengths, and fiber architecture, are few, the available data indicate mixed results in vertebrates. Studies of theoretical bite force in the spotted ratfish (Hydrolagus coliei) show isometry in muscle P_{CSA} (positive allometry of fiber lengths and fiber angles, and variability in muscle mass) and positive allometry in mechanical leverage, resulting in positive allometry in bite-force generation (Huber et al. 2008). Moreover, blacktip sharks (Carcharhinus limbatus) show positive allometry in P_{CSA} and mechanical leverage relative to total length, resulting in positive allometry of theoretical bite-force generation (Huber et al. 2006). Sheepshead (Archosargus probatocephalus) show positive allometry in muscle mass and pharyngeal jaw elements relative to standard body length, resulting in positive allometry of muscle force and bite force across ontogeny (Hernández and Motta 1997). Investigation of prey-crushing force show that Caribbean hogfish (Lachnolaimus maximus) show isometry in pennation angles and positive allometry in muscle mass relative to body mass, allowing larger fish to prey on larger prey items (Wainwright 1987). Furthermore, black drum (Pogonias
cromts) show isometry in muscle $P_{\text{CSA}}$ and positive allometry in mechanical leverage relative to body mass, resulting in positive allometry of prey-crushing ability (Grubich 2005). African catfish (*Clarias gariepinus*) show isometry in muscle mass and positive allometry in $P_{\text{CSA}}$ due to negative allometry in fiber lengths, resulting in positive allometry in theoretical bite-force generation (Herrel *et al.* 2005b). Among reptiles, *Anolis* and *Sceloporus* lizards show positive allometry and isometry in mechanical leverage, respectively, yet both display positive allometry in bite-force generation relative to body mass and head length (Herrel and O’Reilly 2006; Meyers *et al.* 2002). In mammals, the short-tailed opossums (*Monodelphis domestica*) show positive allometry in mechanical leverage, however bite-force generation scales isometrically relative to muscle mass (Thompson *et al.* 2003). All except one of these studies ultimately show positive allometry in bite-force generation, yet scaling of biomechanical properties is not always the same. This suggests that because the feeding apparatus in vertebrates is composed of a complex set of musculoskeletal structures, ontogenetic increases in bite-force generation are not constrained to one universal pattern of morphological modification. While studies in fishes are relatively common, few studies have investigated the ontogeny of feeding biomechanics in tetrapods. More studies would provide an opportunity to assess these concepts from a broader evolutionary framework.

**Implications for Dietary Ontogeny**

Opercula recovered from *S. minor* gut contents show that *S. minor* consume exponentially larger snails throughout ontogeny. This result is consistent with previous dietary studies in *S. minor* (Tinkle 1958; Berry 1975). While turtles in this study did not consume greater numbers of snails across ontogeny, previous work suggests that there is substantial shift in the volume of snails consumed relative to other dietary items between small and large turtles (Tinkle 1958; Berry 1975). The data on the forces required to fracture snails (FF) obtained during the mechanical loading trials indicate that when loaded between the beaks of turtles, FF scaled isometrically relative to snail size (SnL). These results suggest that across ontogeny *S. minor* consume disproportionately larger snails that do not require disproportionately greater force to fracture than smaller snails. Moreover, this result is in contrast with studies by Osenberg and Mittelbach (1989), Hernández and Motta (1997), and Tucker (1997) which found that the
crushing resistance strength of gastropod mollusks increases disproportionately (positive allometry) with changes in size. Nevertheless, when these data were extrapolated to determine the force required to fracture the five largest snails found in the diet (\(A_{FF}\)), these forces scaled with positive allometry relative to skull length and with the same scaling coefficient as bite-force generation (observed and theoretical). These results indicate that there is a close relationship between the force required to fracture dietary items and bite-force generation.

Increases in bite-force generation are known to facilitate increases in dietary breadth (Wainwright 1991; Herrel et al. 1996; Verwaijen et al. 2002; Aguirre et al. 2003; Erickson et al. 2003; Herrel and O’Reilly 2006), however for \(S.\ minor\) an elevation in bite-force generation leads to greater dietary specialization. Turtles in this study consume progressively larger snails that push the limits of their bite-force potential, which suggest that consuming larger snails has some nutritional or energetic advantage for larger turtles over consuming smaller snails. Consequently, developing greater bite forces may increase proficiency in crushing larger prey, thereby increasing the net rate of energy intake when foraging, and presumably enhancing fitness (Anderson et al. 2008). In addition, this result indicates that smaller turtles are incapable of handling and crushing larger snails, and are therefore constrained in their dietary options. Previous studies have also shown that individuals with higher bite forces required less time to consume particular prey items (Herrel et al. 2001; Verwaijen et al. 2002; van der Meij and Bout 2006). Furthermore, because bite force increases with the same scaling relationship as the average force required to fracture the largest dietary items (\(A_{FF}\)), \(S.\ minor\) is apparently trophically limited by the force that can be generated by its musculoskeletal feeding apparatus (Hernández and Motta 1997). For durophagous species, there is often a tight correlation between the prey-crushing ability and the capacity of their morphology to generate bite forces (e.g., Wainwright 1987; Hernández and Motta 1997). For these reasons, the rather extreme morphological changes in the head and beak (Pfaller see Chapter 1), and jaw adductor size and architecture, displayed by \(S.\ minor\) can be linked to the structural properties of their primary prey. The results of this study provide empirical evidence that ontogenetic changes to musculoskeletal morphology and ecology are inextricably linked by the performance of the feeding apparatus. Such results are exceedingly important for establishing a baseline from which to explore the mechanistic and functional issues that underlie the evolution of phenotypic traits (Arnold 1983; Kingsolver and Huey 2003).
Despite the similarity in scaling patterns, $A_{FF}$ values were significantly different than theoretical and observed bite-force values. The mechanical loading trials were designed to test the force required to fracture snails by applying compressive force at a constantly increasing rate until fracture. The results of these trials, however, indicate that fracture under a single, static load occurred at forces that are significantly greater than the force that turtles are capable of generating. Perhaps not surprisingly, these mechanical-loading trials apparently do not necessarily simulate the full breadth of feeding behaviors displayed by *S. minor*. For a turtle of a given size, relatively small snails may be crushed with a single compressive load (similar to the mechanical-loading trials), while large snails may require multiple bite-force loadings. This explanation is consistent with the feeding behavior of *S. minor* (Pfaller personal observations), and other durophagous species (e.g., Dalrymple 1977; Gans *et al.* 1985; Boulding and LaBarbera 1986). For this reason, the forces required to fracture the largest snails in the diet ($A_{FF}$) of *S. minor* does not necessitate bite-force generation of equal magnitude. Instead, failure of larger snails is likely achieved with repeated, subcritical loads, a concept known as fatigue failure (Sandor 1972). Fatigue failure of brittle materials (i.e., gastropod shells) begins when small cracks are initiated or existing cracks are propagated by successive loads, and ends when the structure fails (Boresi *et al.* 1978; Boulding and LaBarbera 1986). Multiple bite-force loadings may allow *S. minor* to exploit small fractures caused during previous loads that compromise the structural integrity of the snail shell, so that snails fracture at lower compressive forces. In other words, the small cracks act as stress concentrators that allow turtles to crush large snails that are apparently outside the range of their bite-force capacity. This explanation suggests that maximum bite force is likely important in foraging efficiency, in which turtles with higher bite forces also require less time to process snails. The potential energetic benefits of achieving greater bite forces and consuming disproportionately more robust prey are beyond the scope of this study, however further investigations into the foraging efficiency (i.e. time to process vs. snail size) would be particularly interesting for elucidating the linkage between variation in maximal bite force and variation in fitness (Anderson *et al.* 2008).
Figure 2.1. Jaw adductor musculature of *Sternotherus minor*. (a) dorsal and (b) lateral view of the Musculus adductor mandibulae externus; (c) lateral view of the M. add. mand. posterior and internus. P. prof., Pars profunda; P. supf., Pars superficialis; P. med., Pars media; M. post., M. posterior; M. pseudo., M. pseudotemporalis; M. ptery., M. pterygoideus.
Figure 2.2. Schematic diagram of the skull and jaw of *Sternotherus minor*. (a) three-dimensional coordinate system used for vector analysis, and the two-dimensional origins and directions of forces acting on the lower jaw relative to the skull. A, Pars superficialis, profunda complex; B, Pars media; C, M. posterior; D, M. pseudotemporalis, pterygoideus complex; E, bite force; J, jaw joint. (b-e) Free-body diagrams of the lower jaw indicating force directions and lever lengths for jaw adductor musculature and bite force. F, muscle force; BF, bite force; IL, in-lever length; OL, out-lever length; jaw joint (shaded circle). (b) Pars superficialis, profunda complex, (c) Pars media, (d) M. posterior, and (e) M. pseudotemporalis, pterygoideus complex.
Figure 2.3. Reduced major axis regression of snail length on operculum length in *Goniobasis* (*n* = 50).

RMA regression slope:
\[ y = 3.83x + 1.55 \]
\[ R^2 = 0.94 \]
Table 2.1. Isometric scaling predictions used for comparisons among morphological variables, and between morphological variables and bite-force generation. Bite-force generation is an area measure because muscle-force generation is determined by the total cross-sectional area of the muscle. Force required to fracture snails is an area measured because the compression strength of the snails is determined by cross-sectional area of the snail shell. Asterisk denotes isometric scaling coefficients that were not used in the present study.

<table>
<thead>
<tr>
<th>Independent Variable ((x))</th>
<th>Angle Measures</th>
<th>Linear Measures</th>
<th>Area Measures</th>
<th>Volume Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear Measures</td>
<td>0.0*</td>
<td>1.0</td>
<td>0.5*</td>
<td>0.34</td>
</tr>
<tr>
<td>Area Measures</td>
<td>0.0*</td>
<td>2.0</td>
<td>1.0</td>
<td>0.67</td>
</tr>
<tr>
<td>Volume Measures</td>
<td>0.0</td>
<td>3.0</td>
<td>1.5*</td>
<td>1.0*</td>
</tr>
</tbody>
</table>

Angle measure: fiber angle. Linear measures: skull length (SL), lever length (IL and OL), and fiber length. Area measures: physiological cross-sectional area, bite-force generation, and force required to fracture snails. Volume measure: muscle mass.
Table 2.2. Descriptive statistics of morphological variables, and percent contribution of each jaw adductor subdivision to total muscle mass, physiological cross-sectional area (P\textsubscript{CSA}), and bite-force generation in \textit{Sternotherus minor}. Theoretical bite-force values assume 30 N cm\textsuperscript{-2} muscle stress and 100% muscle recruitment to represent the maximum possible estimates.

<table>
<thead>
<tr>
<th>Muscle Subdivision</th>
<th>Mass (g)</th>
<th>Fiber Length (cm)</th>
<th>Fiber Angle (deg.)</th>
<th>P\textsubscript{CSA} (cm\textsuperscript{2})</th>
<th>In-lever Length (cm)</th>
<th>Theoretical Bite Force (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{M. adductor mandibulae externus}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. prof. α</td>
<td>1.10 ± 0.18</td>
<td>0.77 ± 0.04</td>
<td>43.0 ± 0.55</td>
<td>0.80 ± 0.10</td>
<td>0.99 ± 0.06</td>
<td>27.47 ± 3.43</td>
</tr>
<tr>
<td>(% of total)</td>
<td>0.10 – 3.0</td>
<td>0.39 – 1.13</td>
<td>39.2 – 49.2</td>
<td>0.16 – 1.92</td>
<td>0.55 – 1.55</td>
<td>5.5 – 66.6</td>
</tr>
<tr>
<td>P. prof. β</td>
<td>0.13 ± 0.023</td>
<td>0.51 ± 0.026</td>
<td>33.4 ± 0.49</td>
<td>0.19 ± 0.03</td>
<td>0.99 ± 0.06</td>
<td>6.52 ± 1.03</td>
</tr>
<tr>
<td>(% of total)</td>
<td>0.012 – 0.46</td>
<td>0.23 – 0.68</td>
<td>28.4 – 37.7</td>
<td>0.027 – 0.56</td>
<td>0.55 – 1.55</td>
<td>0.93 – 19.43</td>
</tr>
<tr>
<td>P. prof. γ</td>
<td>0.48 ± 0.090</td>
<td>0.50 ± 0.026</td>
<td>42.6 ± 0.42</td>
<td>0.57 ± 0.09</td>
<td>0.99 ± 0.06</td>
<td>19.57 ± 3.1</td>
</tr>
<tr>
<td>(% of total)</td>
<td>0.04 – 1.41</td>
<td>0.24 – 0.70</td>
<td>38.7 – 46.6</td>
<td>0.096 – 1.58</td>
<td>0.55 – 1.55</td>
<td>3.3 – 54.83</td>
</tr>
<tr>
<td>P. supf.</td>
<td>0.38 ± 0.072</td>
<td>0.51 ± 0.030</td>
<td>39.7 ± 0.80</td>
<td>0.45 ± 0.06</td>
<td>0.99 ± 0.06</td>
<td>15.45 ± 2.06</td>
</tr>
<tr>
<td>(% of total)</td>
<td>0.04 – 1.27</td>
<td>0.54 – 0.80</td>
<td>34.3 – 48.1</td>
<td>0.11 – 1.12</td>
<td>0.55 – 1.55</td>
<td>3.78 – 38.87</td>
</tr>
<tr>
<td>P. med.</td>
<td>0.12 ± 0.023</td>
<td>0.61 ± 0.030</td>
<td>n/a</td>
<td>0.17 ± 0.026</td>
<td>0.71 ± 0.033</td>
<td>4.19 ± 0.64</td>
</tr>
<tr>
<td>(% of total)</td>
<td>0.013 – 0.40</td>
<td>0.40 – 0.90</td>
<td>n/a</td>
<td>0.033 – 0.48</td>
<td>0.45 – 0.99</td>
<td>0.93 – 10.64</td>
</tr>
</tbody>
</table>

| M. adductor mandibulae posterior |         |                  |                   |                 |                     |                        |
| M. post. | 0.03 ± 0.005 | 0.058 ± 0.027 | n/a | 0.05 ± 0.006 | 0.36 ± 0.017 | 0.62 ± 0.075 |
| (% of total) | 0.004 – 0.12 | 0.35 – 0.90 | n/a | 0.011 – 0.17 | 0.23 – 0.51 | 0.158 – 1.94 |
| M. adductor mandibulae internus |         |                  |                   |                 |                     |                        |
| M. pseud. | 0.11 ± 0.017 | 0.45 ± 0.027 | n/a | 0.21 ± 0.027 | 0.14 ± 0.007 | 1.02 ± 0.13 |
| (% of total) | 0.011 – 0.30 | 0.030 – 0.92 | n/a | 0.036 – 0.51 | 0.071 – 0.20 | 0.16 – 2.28 |
| M. ptery. | 0.05 ± 0.008 | 0.46 ± 0.020 | n/a | 0.094 ± 0.01 | 0.14 ± 0.007 | 0.46 ± 0.049 |
| (% of total) | 0.007 – 0.18 | 0.28 – 0.65 | n/a | 0.022 – 0.29 | 0.071 – 0.20 | 0.098 – 1.30 |

Values are the mean ± standard error (SE) (top), minimum - maximum (middle), and mean percentage ± SE (bottom). P. prof. α, Pars profunda α; P. prof. β, Pars profunda β; P. prof. γ, Pars profunda γ; P. supf., Pars superficialis; P. med., Pars media; M. post., M. posterior; M. pseudo., M. pseudotemporalis; M. ptery., M. pterygoideus.
Table 2.3. Scaling of bite-force generation (observed and theoretical) against skull length in *Sternotherus minor*.

<table>
<thead>
<tr>
<th>Variables (y against x)</th>
<th>$R^2$</th>
<th>Intercept $(a)$</th>
<th>Slope $(b)$</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>$P$-value</th>
<th>Isometric Prediction</th>
<th>Growth Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bite Force against Skull Length</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theoretical BF</td>
<td>0.97</td>
<td>-2.54</td>
<td>2.71</td>
<td>2.54</td>
<td>2.88</td>
<td>&lt; 0.0001</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>Observed BF</td>
<td>0.95</td>
<td>-2.50</td>
<td>2.67</td>
<td>2.50</td>
<td>2.84</td>
<td>&lt; 0.0001</td>
<td>2</td>
<td>P</td>
</tr>
</tbody>
</table>

Significance levels ($\alpha = 0.05$). P-values were corrected using modified t-tests to reflect differences from isometric predictions. For growth types, P = positive allometry.
Figure 2.4. Log-log plots for scaling of observed and theoretical bite-force generation on skull length in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of the observed bite-force data. Regression slopes for observed bite-force data compared to theoretical bite-force estimates modeling 20 N cm\(^{-2}\), 25 N cm\(^{-2}\), and 30 N cm\(^{-2}\) muscle stress, and (a) 80 %, (b) 90 %, and (c) 100 % muscle recruitment. Asterisk indicates slopes that are not statistically different from the observed bite-force slope.
Table 2.4. Summary of paired t-tests comparing theoretical and observed bite-force generation in *Sternotherus minor*. Nine different theoretical models were tested based on three different values for muscle stress (20, 25, and 30 N cm\(^{-2}\)) and three different values from the percentage of muscle recruitment (80, 90, and 100%).

<table>
<thead>
<tr>
<th>Muscle Stress (N cm(^{-2}))</th>
<th>Percentage of Muscle Recruitment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>80</td>
</tr>
<tr>
<td>20</td>
<td>(t_{29} = -15.59)</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.0001)</td>
</tr>
<tr>
<td>25</td>
<td>(t_{29} = -6.08)</td>
</tr>
<tr>
<td></td>
<td>(P &lt; 0.0001)</td>
</tr>
<tr>
<td>30</td>
<td>(t_{29} = 1.68)</td>
</tr>
<tr>
<td></td>
<td>(P = 0.103)</td>
</tr>
</tbody>
</table>
Table 2.5. Scaling of levers. In-lever lengths for jaw adductor muscles against out-lever length in *Sternotherus minor*.

<table>
<thead>
<tr>
<th>Variables</th>
<th>R²</th>
<th>Intercept (a)</th>
<th>Slope (b)</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>P-value</th>
<th>Isometric Prediction</th>
<th>Growth Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-lever Length against Out-lever Length</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL_{sup,pro}</td>
<td>0.99</td>
<td>-0.25</td>
<td>1.01</td>
<td>0.98</td>
<td>1.04</td>
<td>0.25</td>
<td>1.0</td>
<td>I</td>
</tr>
<tr>
<td>IL_{med}</td>
<td>0.92</td>
<td>-0.13</td>
<td>0.80</td>
<td>0.72</td>
<td>0.88</td>
<td>&lt; 0.0001</td>
<td>1.0</td>
<td>N</td>
</tr>
<tr>
<td>IL_{post}</td>
<td>0.96</td>
<td>0.18</td>
<td>0.78</td>
<td>0.72</td>
<td>0.84</td>
<td>&lt; 0.0001</td>
<td>1.0</td>
<td>N</td>
</tr>
<tr>
<td>IL_{pseud,ptery}</td>
<td>0.96</td>
<td>0.63</td>
<td>0.76</td>
<td>0.70</td>
<td>0.82</td>
<td>&lt; 0.0001</td>
<td>1.0</td>
<td>N</td>
</tr>
</tbody>
</table>

Significance levels (α = 0.05). P-values were corrected using modified t-tests to reflect differences from isometric predictions. For growth types, I = isometry, and N = negative allometry. IL_{sup,pro}, Pars superficialis and Pars profunda in-lever length; IL_{med}, Pars media in-lever length; IL_{post}, M. posterior in-lever length; IL_{pseud,ptery}, M. pseudotemporalis and M. pterygoideus in-lever length.
Figure 2.5. Log-log plots for the scaling of in-lever lengths on out-lever length in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of each independent variable. (a) in-lever length of Pars superficialis and Pars profunda (IL\textsubscript{sup,pro}; filled circles) and in-lever length of media (IL\textsubscript{med}; open triangles), and (b) in-lever length of M. posterior (IL\textsubscript{post}; filled circles) and in-lever length of M. pseudotemporalis and M. pterygoideus (IL\textsubscript{pseud,ptery}; open triangles) as a function of out-lever length. I = isometry, and N = negative allometry. Asterisk indicates slope that are significantly different from isometry.
Table 2.6. Scaling of muscle masses. (a) total and isolated muscle mass against skull length, and (b) bite-force generation (observed) against total and isolated muscle mass in *Sternotherus minor*.

<table>
<thead>
<tr>
<th>Variables (y against x)</th>
<th>R²</th>
<th>Intercept ((a))</th>
<th>Slope ((b))</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>P-value</th>
<th>Isometric Prediction</th>
<th>Growth Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(a) Muscle Mass against Skull Length</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. prof. (\alpha)</td>
<td>0.97</td>
<td>-4.25</td>
<td>3.90</td>
<td>3.66</td>
<td>4.14</td>
<td>&lt; 0.0001</td>
<td>3.0</td>
<td>P</td>
</tr>
<tr>
<td>P. prof. (\beta)</td>
<td>0.94</td>
<td>-4.96</td>
<td>3.79</td>
<td>3.43</td>
<td>4.15</td>
<td>&lt; 0.0001</td>
<td>3.0</td>
<td>P</td>
</tr>
<tr>
<td>P. prof. (\gamma)</td>
<td>0.98</td>
<td>-5.04</td>
<td>4.17</td>
<td>3.93</td>
<td>4.41</td>
<td>&lt; 0.0001</td>
<td>3.0</td>
<td>P</td>
</tr>
<tr>
<td>P. supf.</td>
<td>0.95</td>
<td>-4.87</td>
<td>4.01</td>
<td>3.67</td>
<td>4.35</td>
<td>&lt; 0.0001</td>
<td>3.0</td>
<td>P</td>
</tr>
<tr>
<td>P. med.</td>
<td>0.95</td>
<td>-4.90</td>
<td>3.73</td>
<td>3.41</td>
<td>4.05</td>
<td>&lt; 0.0001</td>
<td>3.0</td>
<td>P</td>
</tr>
<tr>
<td>M. post</td>
<td>0.89</td>
<td>-3.59</td>
<td>3.18</td>
<td>2.78</td>
<td>3.57</td>
<td>0.185</td>
<td>3.0</td>
<td>I</td>
</tr>
<tr>
<td>M. pseudo.</td>
<td>0.96</td>
<td>-3.50</td>
<td>3.45</td>
<td>3.18</td>
<td>3.72</td>
<td>0.001</td>
<td>3.0</td>
<td>P</td>
</tr>
<tr>
<td>M. ptery.</td>
<td>0.92</td>
<td>-3.26</td>
<td>3.09</td>
<td>2.76</td>
<td>3.42</td>
<td>0.30</td>
<td>3.0</td>
<td>I</td>
</tr>
<tr>
<td><strong>Total Mass</strong></td>
<td>0.98</td>
<td>-3.85</td>
<td>3.87</td>
<td>3.66</td>
<td>4.09</td>
<td>&lt; 0.0001</td>
<td>3.0</td>
<td>P</td>
</tr>
<tr>
<td><strong>(b) Bite Force against Muscle Mass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. prof. (\alpha)</td>
<td>0.97</td>
<td>0.16</td>
<td>0.78</td>
<td>0.75</td>
<td>0.85</td>
<td>&lt; 0.0001</td>
<td>0.67</td>
<td>P</td>
</tr>
<tr>
<td>P. prof. (\beta)</td>
<td>0.92</td>
<td>0.85</td>
<td>0.82</td>
<td>0.73</td>
<td>0.91</td>
<td>0.001</td>
<td>0.67</td>
<td>P</td>
</tr>
<tr>
<td>P. prof. (\gamma)</td>
<td>0.94</td>
<td>0.54</td>
<td>0.75</td>
<td>0.68</td>
<td>0.82</td>
<td>0.013</td>
<td>0.67</td>
<td>P</td>
</tr>
<tr>
<td>P. supf.</td>
<td>0.93</td>
<td>0.56</td>
<td>0.78</td>
<td>0.70</td>
<td>0.86</td>
<td>0.0035</td>
<td>0.67</td>
<td>P</td>
</tr>
<tr>
<td>P. med.</td>
<td>0.91</td>
<td>0.87</td>
<td>0.84</td>
<td>0.74</td>
<td>0.94</td>
<td>0.0007</td>
<td>0.67</td>
<td>P</td>
</tr>
<tr>
<td>M. post</td>
<td>0.89</td>
<td>0.30</td>
<td>0.98</td>
<td>0.85</td>
<td>1.11</td>
<td>&lt; 0.0001</td>
<td>0.67</td>
<td>P</td>
</tr>
<tr>
<td>M. pseudo.</td>
<td>0.93</td>
<td>-0.07</td>
<td>0.91</td>
<td>0.82</td>
<td>1.00</td>
<td>&lt; 0.0001</td>
<td>0.67</td>
<td>P</td>
</tr>
<tr>
<td>M. ptery.</td>
<td>0.86</td>
<td>0.067</td>
<td>1.01</td>
<td>0.87</td>
<td>1.15</td>
<td>&lt; 0.0001</td>
<td>0.67</td>
<td>P</td>
</tr>
<tr>
<td><strong>Total Mass</strong></td>
<td>0.96</td>
<td>-0.129</td>
<td>0.81</td>
<td>0.75</td>
<td>0.87</td>
<td>&lt; 0.0001</td>
<td>0.67</td>
<td>P</td>
</tr>
</tbody>
</table>

Significance levels \((\alpha = 0.05)\). P-values were corrected using modified t-tests to reflect differences from isometric predictions. For growth types, P = positive allometry, and I = isometry. P. prof. \(\alpha\), Pars profunda \(\alpha\); P. prof. \(\beta\), Pars profunda \(\beta\); P. prof. \(\gamma\), Pars profunda \(\gamma\); P. supf., Pars superficialis; P. med., Pars media; M. post., M. posterior; M. pseudo., M. pseudotemporalis; M. ptery., M. pterygoideus.
Figure 2.6. Log-log plots for the scaling of isolated muscle masses on skull length in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of each independent variable. (a) Pars profunda α, (b) Pars profunda β, (c) Pars profunda γ, (d) Pars superficialis, (e) Pars media, (f) M. posterior, (g) M. pseudotemporalis, and (h) M. pterygoideus. P = positive allometry, and I = isometry. Asterisk indicates slope that are significantly different from isometry.
Figure 2.7. Log-log plots for the scaling of total M. adductor mandibulae mass on skull length in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of each independent variable. $P =$ positive allometry. Asterisk indicates slope that is significantly different from isometry.
Figure 2.8. Log-log plots for the scaling of bite force on isolated muscle masses in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of each independent variable. (a) Pars profunda $\alpha$, (b) Pars profunda $\beta$, (c) Pars profunda $\gamma$, (d) Pars superficialis, (e) Pars media, (f) M. posterior, (g) M. pseudotemporalis, and (h) M. pterygoideus. $P =$ positive allometry. Asterisk indicates slope that are significantly different from isometry.
Figure 2.9. Log-log plots for the scaling of bite force on total M. adductor mandibulae mass in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of each independent variable. P = positive allometry. Asterisk indicates slope that is significantly different from isometry.
Table 2.7. Scaling of muscle architecture and physiological cross-sectional area. (a) average fiber length against isolated and total muscle mass, (b) average fiber angle against isolated and total muscle mass, and (c) bite-force generation (observed) against isolated and total physiological cross-sectional area in *Sternotherus minor*.

<table>
<thead>
<tr>
<th>Variables (y against x)</th>
<th>R²</th>
<th>Intercept (α)</th>
<th>Slope (b)</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>P-value</th>
<th>Isometric Prediction</th>
<th>Growth Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Fiber Length against Muscle Mass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. prof. α</td>
<td>0.94</td>
<td>1.38</td>
<td>0.27</td>
<td>0.24</td>
<td>0.30</td>
<td>&lt; 0.0001</td>
<td>0.34</td>
<td>N</td>
</tr>
<tr>
<td>P. prof. β</td>
<td>0.73</td>
<td>1.41</td>
<td>0.30</td>
<td>0.24</td>
<td>0.36</td>
<td>0.09</td>
<td>0.34</td>
<td>I</td>
</tr>
<tr>
<td>P. prof. γ</td>
<td>0.87</td>
<td>1.32</td>
<td>0.26</td>
<td>0.22</td>
<td>0.30</td>
<td>&lt; 0.0001</td>
<td>0.34</td>
<td>N</td>
</tr>
<tr>
<td>P. supf.</td>
<td>0.91</td>
<td>1.29</td>
<td>0.29</td>
<td>0.26</td>
<td>0.32</td>
<td>0.0003</td>
<td>0.34</td>
<td>N</td>
</tr>
<tr>
<td>P. med.</td>
<td>0.87</td>
<td>1.55</td>
<td>0.25</td>
<td>0.22</td>
<td>0.28</td>
<td>&lt; 0.0001</td>
<td>0.34</td>
<td>N</td>
</tr>
<tr>
<td>M. post</td>
<td>0.83</td>
<td>1.36</td>
<td>0.29</td>
<td>0.24</td>
<td>0.34</td>
<td>0.019</td>
<td>0.34</td>
<td>I</td>
</tr>
<tr>
<td>M. pseudo.</td>
<td>0.68</td>
<td>1.08</td>
<td>0.30</td>
<td>0.24</td>
<td>0.36</td>
<td>0.110</td>
<td>0.34</td>
<td>I</td>
</tr>
<tr>
<td>M. ptery.</td>
<td>0.61</td>
<td>1.22</td>
<td>0.28</td>
<td>0.21</td>
<td>0.35</td>
<td>0.0394</td>
<td>0.34</td>
<td>I</td>
</tr>
<tr>
<td>(b) Fiber Angles against Muscle Mass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. prof. α</td>
<td>0.86</td>
<td>1.52</td>
<td>0.062</td>
<td>0.054</td>
<td>0.070</td>
<td>&lt; 0.0001</td>
<td>0.0</td>
<td>P</td>
</tr>
<tr>
<td>P. prof. β</td>
<td>0.78</td>
<td>1.46</td>
<td>0.075</td>
<td>0.061</td>
<td>0.089</td>
<td>&lt; 0.0001</td>
<td>0.0</td>
<td>P</td>
</tr>
<tr>
<td>P. prof. γ</td>
<td>0.59</td>
<td>1.56</td>
<td>0.046</td>
<td>0.036</td>
<td>0.056</td>
<td>&lt; 0.0001</td>
<td>0.0</td>
<td>P</td>
</tr>
<tr>
<td>P. supf.</td>
<td>0.84</td>
<td>1.47</td>
<td>0.094</td>
<td>0.080</td>
<td>0.110</td>
<td>&lt; 0.0001</td>
<td>0.0</td>
<td>P</td>
</tr>
<tr>
<td>(c) Bite Force against P&lt;sub&gt;CSA&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. prof. α</td>
<td>0.97</td>
<td>-0.45</td>
<td>1.15</td>
<td>1.07</td>
<td>1.23</td>
<td>0.0004</td>
<td>1.0</td>
<td>P</td>
</tr>
<tr>
<td>P. prof. β</td>
<td>0.79</td>
<td>0.32</td>
<td>1.12</td>
<td>0.92</td>
<td>1.32</td>
<td>0.120</td>
<td>1.0</td>
<td>I</td>
</tr>
<tr>
<td>P. prof. γ</td>
<td>0.89</td>
<td>-0.01</td>
<td>1.01</td>
<td>0.89</td>
<td>1.13</td>
<td>0.434</td>
<td>1.0</td>
<td>I</td>
</tr>
<tr>
<td>P. supf.</td>
<td>0.89</td>
<td>-0.18</td>
<td>1.08</td>
<td>0.94</td>
<td>1.22</td>
<td>0.131</td>
<td>1.0</td>
<td>I</td>
</tr>
<tr>
<td>P. med.</td>
<td>0.87</td>
<td>0.40</td>
<td>1.09</td>
<td>0.93</td>
<td>1.25</td>
<td>0.135</td>
<td>1.0</td>
<td>I</td>
</tr>
<tr>
<td>M. post</td>
<td>0.79</td>
<td>-0.49</td>
<td>1.33</td>
<td>1.11</td>
<td>1.55</td>
<td>0.0027</td>
<td>1.0</td>
<td>P</td>
</tr>
<tr>
<td>M. pseudo.</td>
<td>0.85</td>
<td>-0.99</td>
<td>1.18</td>
<td>1.02</td>
<td>1.36</td>
<td>0.0160</td>
<td>1.0</td>
<td>P</td>
</tr>
<tr>
<td>M. ptery.</td>
<td>0.74</td>
<td>-0.77</td>
<td>1.27</td>
<td>1.03</td>
<td>1.51</td>
<td>0.0160</td>
<td>1.0</td>
<td>P</td>
</tr>
<tr>
<td><strong>Total Mass</strong></td>
<td>0.93</td>
<td>-1.023</td>
<td>1.09</td>
<td>0.97</td>
<td>1.33</td>
<td>0.163</td>
<td>1.0</td>
<td>I</td>
</tr>
</tbody>
</table>

Significance levels (α = 0.05). P-values were corrected using modified t-tests to reflect differences from isometric predictions. For growth types, P = positive allometry, I = isometry, and N = negative allometry. P. prof. α, Pars profunda α; P. prof. β, Pars profunda β; P. prof. γ, Pars profunda γ; P. supf., Pars superficialis; P. med., Pars media; M. post., M. posterior; M. pseudo., M. pseudotemporalis; M. ptery., M. pterygoideus.
Figure 2.10. Log-log plots for the scaling of average fiber length and pennation angle on isolated muscle masses in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of each independent variable. (a, b) Pars profunda α, (c, d) Pars profunda β, (e, f) Pars profunda γ, (g, h) Pars superficialis. P = positive allometry, I = isometry, and N = negative allometry.
Figure 2.11. Log-log plots for the scaling of bite force on isolated physiological cross-sectional area (P\(_{\text{CSA}}\)) in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of each independent variable. (a) Pars profunda \(\alpha\), (b) Pars profunda \(\beta\), (c) Pars profunda \(\gamma\), (d) Pars superficialis, (e) Pars media, (f) M. posterior, (g) M. pseudotemporalis, and (h) M. pterygoideus. P = positive allometry, and I = isometry. Asterisk indicates slope that are significantly different from isometry.
Figure 2.12. Log-log plots for the scaling of bite force on total M. adductor mandibulae physiological cross-sectional area (P_{CSA}) in *Sternotherus minor*. Solid lines, reduced major axis regressions for the data; dashed lines, scaling predictions based on isometric growth set to cross data lines at the mean values of each independent variable. I = isometry. Asterisk indicates slope that is significantly different from isometry.
Figure 2.13. Dietary data against skull length. (a) Number of opercula per turtle and (b) average estimated snail length. (b) y-bars represent the estimated minimum and maximum size of snail found in the diet of each turtle.
Figure 2.14. Reduced major axis regression of the force required to fracture snails (FF) on snail length of *Goniobasis* (*n* = 77). (a) Untransformed regression used to predict forces required to fracture snails in digestive tracts, and (b) log-log plot used to assess the scaling of force required to fracture on snail length.
Figure 2.15. Log-log plots for reduced major axis regressions of (a) the average force required to fracture the five largest snails per turtle (A_{FF}) and (b) A_{FF} (open circles, dotted line), observed bite force (filled diamonds, black line), and theoretical bite force (filled triangles, dashed lines) on skull length. Theoretical bite-force values assume 30 N cm^{-2} muscle stress and 100% muscle recruitment to represent the maximum possible estimates. Asterisk indicates slope that are significantly different from isometry.
CHAPTER 3

GROWTH AND BITE-FORCE GENERATION IN THE LOGGERHEAD MUSK TURTLE, *STERNOTHERUS MINOR*: AN ASSESSMENT USING SKELETOCHRONOLOGY

Although information on longevity and growth patterns is imperative to gain a more comprehensive understanding of the ecology and evolution of reptiles, these aspects of their biology have not been extensively studied (Erickson 2003). Some of this stems from the logistic limitations of monitoring wild populations (Gibbons 1976; Zug 1990). Consequently, our understanding of age-related life history, growth, and performance in most reptiles has been limited (Erickson 2003).

This limitation has been somewhat remedied, however, by the practical applications of using skeletal growth lines to study development in ectothermic vertebrates (Castanet 1994; Smirina 1994). Because bones form layers of predictable periodicity as they grow (Klevezal 1996), counts and measurements of these structures allow for postmortem assessments of longevity and relative growth rates throughout ontogeny (Erickson et al. 2001). Moreover, results indicate that growth lines are mostly formed annually as a result of restricted growth associated with seasonal biotic and abiotic differences (Snover and Rhodin 2008). Since its inception, age determination via skeletochronology has been utilized and validated in many reptilian systems (e.g., Parham and Zug 1997; Waye and Gregory 1998; Coles et al. 2001; Erickson et al. 2001; Erickson 2003; Snover and Hohn 2004; Curtin 2006; Snover et al. 2007; Curtin et al. 2008).

The advent of skeletochronology has provided an excellent tool to elucidate the relative rates of growth in morphological structures, however very few studies have used age estimates to address the rates of change in organismal performance and none have investigated this in reptilian systems (Binder and Van Valkenburgh 2000; Thompson et al. 2003). Performance is the ability of an organism to accomplish ecologically-relevant tasks (Wainwright 1994) and is
explicitly linked to the morphology of the musculoskeletal system. Moreover, the performance of the feeding apparatus in particular has profound impacts on survival through its role in procuring, processing, and ingesting food resources (Emerson and Bramble 1993). As organisms grow juveniles are expected to perform differently because changes in size necessitate changes in physical capacities (Hill 1950; Schmidt-Nielson 1975). The consequences of changes in size for organismal design have long been of interest to biologists (e.g., Huxley 1924, 1932; Hill 1950; Gould 1966, 1971; Schmidt-Nielsen 1975), however these changes are rarely addressed in terms of age from which the timing and rates of changes in performance can be revealed.

For this work, I will determine the ages of an ontogenetic series of loggerhead musk turtles, *Sternotherus minor*, using skeletochronology. Thirty of these animals were sacrificed for previous analyses of bite-force generation, feeding biomechanics and dietary composition, thus they provided an excellent opportunity to evaluate the relationships between these parameters and age. My specific goals were to: (1) compare the growth patterns determined in this study with a previous mark-recapture study conducted on the same *S. minor* population (Onorato 1996), (2) compare growth patterns of body size, head size, and bite force during growth, and (3) determine the relationships between age, bite-force generation, and dietary force requirements.

Materials and Methods

Experimental Animals

On 26 April 2008, an ontogenetic series of 30 *Sternotherus minor* [yearlings (26 g, 53.2 mm carapace length) to adults (331 g, 126.5 mm carapace length)] were hand captured while snorkeling from the Rainbow River, Marion Co., Florida, USA. Upon capture, specimens were held in shaded containers prior to data collection. At the field site, all individuals were measured for standard external morphometrics [carapace length (CL) and head length (HL); for descriptions see Pfaller Chapter 1] and subaerial bite-force generation. Animals were then transported to a nearby veterinary clinic and euthanized with an intravenous overdoes of pentobarbital sodium (390 mg/ml; 0.5 ml/individual) in accordance with the guidelines of the Institutional Animal Care and Use Committee of Florida State University (ACUC Protocol #
Specimens were placed on ice and transported to the histology laboratory at Florida State University (Tallahassee, FL) where femora and digestive tracts were excised.

Bite-Force Generation

To measure bite-force generation (BF) from the ontogenetic series of 30 *S. minor*, I used the same protocol and apparatus as described by Pfaller (see Chapter 1). The apparatus was composed of a high impedance load cell (Kistler Instrument Corp., Type 9212) sandwiched between two stainless-steel cantilever beams, such that the voltage output routed through a charge amplifier (Kistler Instrument Corp., Type 5995A) was proportional to the compressive force applied to the pair of beams. Two bite-plate configurations were used depending on the size of turtle being tested (3 mm gape configuration for turtles less than 100 g; 5 mm gape configuration from turtles greater than 100 g). Animals were restrained, and appropriate bite plates were placed unilaterally between the jaws, centered mesio-distally at the trough of the crushing surface along the lower beak. Each individual was measured for subaerial bite-force generation during a sequence of three bite-force trials separated by approximately two minutes. The highest of the three bite forces registered by an individual was treated in post hoc analyses as the maximum bite force.

Skeletochronology

Femora were excised from the left side of each animal, cleaned of excess tissue, and individually mounted in epoxy resin. Cross-sections were removed from the diaphysis of each femur immediately distal to the deltopectoral crest (Parham and Zug 1997). The resin-mounted bone sections (0.04 – 0.07 mm) were cut using a slow-speed saw (Isomet 1000, Buehler, Lake Bluff, IL) fitted with a diamond-tipped blade and placed in a decalcifying agent (Formical-4, Decal Chemical Corp., Tallman, NY) for approximately 2 weeks. Sections were then removed from the decalcifying agent and stained using a modified hematoxylin and eosin staining protocol (Thompson 1966; Sheehan and Hrapchak 1987). For each sample, the staining protocol went as follows: buffered formalin (1 min), Harris Hematoxylin (Accustain® Sigma-Aldrich, St. Louis, MO) (2 min.), tap water (exchanged until water remained clear with agitation), eosin (Accustain® Sigma-Aldrich, St. Louis, MO) (2 min.), tap water (exchanged until water remained clear with agitation), 50 % alcohol (1 min.), 70 % alcohol (1 min.), 80 % alcohol (1 min.), 95 %
alcohol (4 min.), tap water (1 min.). At each stage, samples were completely immersed in solution and slightly agitated. Upon completion of the last stage of the staining protocol, samples were gently removed from resin and sandwiched between two glass slides. Samples were viewed at 20 to 400 x magnification with a polarizing petrographic microscope (Olympus SZX12, Olympus Optical Co., Japan) and digital photographs were taken under magnification (Olympus DP11 and Olympus U-CMAD3, Olympus Optical Co., Japan).

For each specimen, annual growth rings were counted, and short-axis measurements (ocular micrometer to the nearest micron) were taken of each growth line, as well as femoral and cortical diameters. Because femora typically show perimedullary resorption with size (Enlow 1969), a sequential stacking method was used to estimate the number of growth marks that were lost to this process (Erickson et al. 2004). This technique involved comparing the short-axis diameters of growth rings, femora, and cortices between small and large turtles. Growth rings were assumed to have been lost in large turtles if the femoral diameter of a small turtle was less than the cortical diameter of a large turtle. Consequently, the number of growth lines in the small turtle were added to the number of visible growth lines to estimate the age of the larger turtle.

**Force to Fracture Experiments**

The force required to fracture (FF) snails [Goniobasis (= Elimia)] commonly consumed by *Sternotherus minor* (e.g., Tinkle 1958; Berry 1975) was determined through a series of mechanical loading experiments. *Goniobasis* snails are common prey items of *S. minor* throughout its range and likely represent the most robust prey in the diet. First, the sizes of snails consumed by each individual were determined by recovering opercula from the digestive tracts. Digestive tracts (esophagus to rectum) were excised and contents were removed. All detectable opercula were recovered and measured for maximum length (OL) from digital photographs (ImageJ v. 1.40, National Institute of Health, Bethesda, MD). Snail sizes were reconstructed based on the relationship between OL and maximum length (SnL) from a sample of 50 wild caught snails. Operculum length was a strong predictor of SnL ($R^2 = 0.94$, t$_{48} = 26.3$, P < 0.0001: SnL = 1.55 + 3.83*OL; Figure 2.3), therefore it was used as a proxy for estimating snail size.
Using a mechanical loading frame (Model 312.31, Materials Testing Solutions, Eden Prairie, MN), the FF for *Goniobasis* was tested within the jaws of three different sized turtles (large - 306 g; medium – 174 g; small – 85 g). Turtle heads were removed from specimens used for the skeletochronological component of the study. For each turtle, a size series of *Goniobasis* representing the range of snails consumed by each turtle were tested. Turtle heads were aligned upside down in a custom-built frame such that the head was braced laterally and the jaw joint was restrained to resist ventral disarticulation. Live *Goniobasis* collected from the wild were aligned between the jaws in a consistent orientation: the widest whorl contacting the flat crushing surfaces of the upper and lower beak. The regression equation (RMA; Figure 2.14a) of FF on snail size was used to extrapolate the force necessary to fracture snails of estimated dimensions (based on operculum length) found in the digestive tracts of turtles. For each turtle, the FF of the five largest snails found in the diet was averaged ($A_{FF}$).

**Statistical Analyses**

Growth patterns were established by plotting values of carapace length and head length against age and fitting a von Bertalanffy growth curve using maximum likelihood and a normal error distribution (von Bertalanffy 1938; Fabens 1965):

\[ X = a(1 - e^{-bt}), \]

where $X$ is the variable (CL or HL) under consideration, $a$ is the estimated asymptotic value of $X$, $b$ is a parameter related to $X$ at hatching, $e$ is the base of the Naperian logarithms, $k$ is the intrinsic growth rate, and $t$ is age in years. To compare the growth patterns found in this study with patterns found by other growth studies on *S. minor* (Onorato 1996), raw values of carapace length were used. To compare growth patterns between carapace length (CL) and head length (HL) proportional values were used. For each variable, raw values were divided by the maximum value for that variable and fitted against age.

Similarly, growth patterns were established by plotting bite force and the average force to fracture the five largest snails ($A_{FF}$) in each turtle’s diet against age and fitting a logistic growth curve:

\[ X = (ae^{tb}) / (a + e^{tb} - 1), \]

where $X$ is the variable (BF or $A_{FF}$) under consideration, $a$ is the estimated asymptotic value of $X$, $b$ is a parameter related to $X$ at hatching, $e$ is the base of the Naperian logarithms, and $t$ is age.
in years. Proportional values were compared for these variables. Significance values were determined by comparing maximum likelihood parameters with chi-square tests. All statistics were performed using R for Windows 2.8.1 (The R Foundation for Statistical Computing, 2008).

**Results**

Carapace length (CL) fit a von Bertalanffy growth curve (CL = 158.17 \(1 - 0.758e^{(-0.09t)}\); Figure 3.1). This von Bertalanffy growth curve was not significantly different from the growth curve reported by Onorato (1996) \((df = 3, P = 0.17; \text{Figure 3.1})\). Proportion of maximum CL and head length (HL) both fit a von Bertalanffy growth curves \((CL = 1.28 \ (1 - 0.780e^{(-0.08t)}); HL = 1.13 \ (1 - 0.758e^{(-0.09t)}); \text{Figure 3.2})\). Proportion of maximum bite force fit a logistic growth curve \((BF = (1.000e^{(t0.6)})/(1.000 + e^{(t0.06) - 1}); \text{Figure 3.2})\). The von Bertalanffy growth curves for the proportion of maximum carapace length (CL) and head length (HL) were statistically different from each other \((df = 3, P < 0.0001; \text{Figure 3.2})\), and both were statistically different from the logistic growth curve for the proportion of maximum bite force \((CL: \ df = 3, P < 0.0001; \text{HL: } df = 3, P < 0.0001; \text{Figure 3.2})\). The logistic growth curve for the proportion of maximum average force required to fracture the five largest snails in the diet \((A_{FF} = (1.000e^{(t0.7)})/(1.000 + e^{(t0.7) - 1}); \text{Figure 3.3})\) was not significantly different from the logistic growth curve for the proportion of maximum bite force \((df = 3, P = 0.532; \text{Figure 3.3})\).

**Discussion**

**Comparison With Onorato (1996)**

The results of this skeletochronological study validate the growth pattern estimated by the mark-recapture study conducted by Onorato (1996) for *Sternotherus minor* inhabiting the Rainbow River, Florida. The von Bertalanffy growth curves developed by this study and that of Onorato (1996) are somewhat different in structure, but are not statistically different. The results from this study suggest that growth in these turtles at this location may be indeterminate. The predicted maximum size as estimated by the asymptote for this study \((a = 152.17 \text{ mm})\) is just
beyond that maximum documented size for these animals (145 mm; Enge and Foster 1986; Camp 1986) and adds support to the observed growth pattern. This is slightly different from the growth pattern predicted by the Onorato (1996) curve, in which growth of the carapace is predicted to terminate near ~130 mm before death. This discrepancy may be the result of the difficulty involved in estimating carapace growth rates for turtles that are near maximum size or a result of growth rates being highly variable (Cox et al. 1991). Nevertheless, these curves are similar and provide some validation for both skeletochronological techniques and mark-recapture growth studies.

The annual deposition of skeletal growth marks in chelonians have been validated by studies of known-aged animals (e.g., Snover and Hohn 2004; Curtin 2006, 2008) and mark-recapture studies (Castanet and Cheylan 1979; Klinger and Musick 1992; Coles et al. 2001; Snover 2007). The results of the present study provide validation for aging through skeletochronology and for the effectiveness of the sequential back-calculating method used to estimate lost growth lines. Back-calculation techniques applied to turtles have rarely been validated (Snover and Rhodin 2008).

**Age, Bite Force, and Dietary Ontogeny**

Ontogenetic growth in *S. minor* is characterized by positive allometry in head size relative to carapace length (Pfaller see Chapter 1). The results of this study show that the onset of this developmental increase is early in the ontogeny of these animals. Cranial expansion does not show a sudden divergence from growth of the carapace, instead cranial hypertrophy in these animals is achieved by a continuous pattern of enlargement that is present at all stages of development. While not shown in Figure 3.2, head width and head depth tracked at similar rates to the growth of head length. These elevated rates in cranial dimensions facilitate the development of progressively larger muscle masses required for bite-force generation used in durophagy (Pfaller see Chapter 2).

While these results provide additional support for the von Bertalanffy model as a suitable curve for the growth of linear measures in reptiles (e.g., Bjorndal and Bolten 1988; Cox et al. 1991), the von Bertalanffy growth curve did not accurately describe the development of bite-force generation. Instead, bite-force generation shows a logistic (S-shaped) growth pattern, in which growth begins slowly, accelerates, slows again, and then reaches some asymptotic value.
This growth pattern is characteristic of post-natal changes in mass among vertebrates (Erickson et al. 2004). This result emphasizes the dramatic change in bite-force generation that these animals undergo during their development. Moreover, these results suggest that bite force has some upper limit, perhaps a result of senescence, at which turtles can no longer elevate their bite-force capacity. Bite force appears to level off after approximately 15 years of growth; however it appears that head and carapace length continue to grow after this age. My interpretation is that this result reflects the limitations of comparing various model fitting equations. The von Bertalanffy and logistic growth curves are fundamentally different (Parham and Zug 1997). Because this a pioneering attempt to fit performance data to morphological growth curves, it is not surprising that these equations do not represent the bite-force data very well. A logistic growth curve fit the bite-force data better than the von Bertalanffy, however it does not appear to be the best overall representation of the bite-force data. Whereas the logistic equation fit bite-force values between the ages of 0 and 10, it seems to misrepresent the changes in bite force among adult greater that 10 years of age. These old adults do show a decline in the rate of increase in bite-force generation, however it does not appear that bite force actually reaches a true asymptote. An additional sample representing individuals around 20 years of age may help to verify the accuracy of this model prediction. Moreover, further investigations of the effect of age on performance traits would be particularly insightful for determining the consistency of these results.

The logistic growth pattern for the force required to fracture the largest snails found in the diet was not significantly different than the logistic growth pattern for bite-force generation. This result provides additional support for the fact that there is strong selection for increased bite-force generation throughout the ontogeny of these animals. Moreover, because bite force tracks closely with changes in dietary force requirements, these turtles are continuously limited in their snail-crushing ability by the capacity of their feeding apparatus to generate bite forces. Along with Pfaller (Chapter 2), the results of this study provide empirical evidence that ontogenetic changes to morphology and ecology are inextricably linked by the performance of the feeding apparatus.

Such striking correspondence between the development of bite-force generation and the forces required to process dietary items suggests that the feeding apparatus of S. minor in this study population may be responding plastically to increased mechanical stress incurred during
feeding (Dalrymple 1977). As turtles grow (age) and process more durable prey, the mechanical stresses exerted on the musculoskeletal system may influence the underlying genetic propensity for craniofacial and musculoskeletal hypertrophy (Emerson and Bramble 1993) and elevate bite-force generation. In this case, individual feeding habits and/or environmental conditions may modulate phenotypic changes to a greater extent than the organism’s genotype (Schlichting and Pigliucci 1998; Garland and Kelly 2006). Work on *S. minor* in north Florida by Berry (1975) supports this conclusion. In some localities, *S. minor* fails to develop its specialized mollusk-crushing adaptations; head width in these populations is significantly smaller than other populations (Berry 1975). Moreover, these locations support dramatically smaller populations of *Goniobasis* snails, and *S. minor* inhabits a more generalized foraging niche (Berry 1975). While this may be attributed to local adaptation, in which selection for cranial hypertrophy and elevated bite-force generation has been relaxed through time, these findings also support the possibility that ontogenetic phenotypic plasticity may be shaping the differences in morphology and ecology of *S. minor* throughout its range.

Ontogenetic phenotypic plasticity has been suggested to explain cranial hypertrophy in other durophagous turtles [*Apalone (= Trionyx)* (Dalrymple 1977); *Graptemys*, and *Emydura* (Emerson and Bramble 1993)], however the extent of its influence has never been tested empirically. Long-lived taxa, like turtles, might benefit from the capacity of their musculoskeletal system to respond plastically to meet changing environmental conditions. Diet can induce plastic responses in fish (e.g., Greenwood 1965; Meyer 1987; Wintzer and Motta 2005) and mammals (e.g., Beecher and Corruccini 1981a, b; Corruccini and Beecher 1982; Corruccini and Lee 1984; Myers *et al*. 1996), however very few studies have investigated phenotypic plasticity in reptiles (except see Schuett *et al*. 2005). The feeding apparatus of durophagous turtles and its ability to generate bite forces may provide an opportunity to elucidate the functional role of phenotypic plasticity in long-lived reptiles.
Figure 3.1. Comparison between von Bertalanffy growth curve estimates for carapace length (CL) in *Sternotherus minor* from this study ($n = 30$; black line, open circles) and Onorato (1996) (dashed line). $X$, carapace length; $a$, estimated asymptotic value of carapace length; $b$, parameter related to CL at hatchling; $k$, intrinsic growth rate; $t$, age in years. These growth curves are not statistically different (df = 3, $P = 0.17$).
Figure 3.2. Comparison between von Bertalanffy growth curve estimates for the proportion of maximum head length (HL; black line, closed circles), and carapace length (CL; dashed line, open circles), and logistic growth curve estimates for the proportion of maximum bite force (BF; grey line, grey triangles), for *Sternotherus minor* (*n* = 30). 

X, carapace length (CL), head length (HL), or bite force (BF); a, estimated asymptotic value of X; b, parameter related to X at hatchling; k, intrinsic growth rate; t, age in years. Superscript letters designate significant differences based on alpha-value of 0.05. All curves are statistically different.
Figure 3.3. Logistic growth curve estimates for the proportion of maximum $A_{FF}$ (dashed line, open circle) and BF (solid line, closed circle). $A_{FF}$, average force to fracture the five largest snails in diet; BF, bite force. These growth curves are not statistically different (df = 2, $P = 0.932$).

Logistic: $X = \frac{ae^{tb}}{a + e^{tb} - 1}$

$BF = \frac{1.000e^{t0.6}}{1.000 + e^{t0.6} - 1}$

$A_{FF} = \frac{1.000e^{t0.7}}{1.000 + e^{t0.7} - 1}$
CONCLUSIONS

Ontogenetic growth can profoundly affect the ability of organisms to perform ecologically-relevant feeding tasks that ultimately impact survival. Bite-force generation is exceedingly important for vertebrates that process and consume durable prey (i.e. durophy). The ontogeny of bite-force generation has been studied in a wide range of tetrapod vertebrates and results typically show a similar pattern of significant positive allometry relative to head and body dimensions. Very few detailed, quantitative studies, however, have examined the musculoskeletal biomechanics of an ontogenetic series to explain this disproportionate increase in bite-force generation. I studied the ontogeny of bite-force generation and feeding biomechanics in the loggerhead musk turtle, *Sternotherus minor*. This species undergoes dramatic morphological changes that reflect a strong tendency towards increased durophy, which was believed to correlate with a relative increase in bite-force generation. Therefore, the objective of this research was to determine the functional relationships between the ontogeny of craniofacial morphology, bite-force generation, and durophy. First, I quantified the degree of craniofacial hypertrophy and its relationship to bite-force generation. Second, I developed an accurate biomechanical model for bite-force generation and used it as a tool to investigate which musculoskeletal traits were important for bite-force generation and durophy. And third, I determined age via skeletochronology and evaluated the rates of change in morphology, performance, and dietary robustness.

Ontogenetic growth in *S. minor* was characterized by allometric increases (i.e. positive allometry) in the dimensions of the head and beak relative to indices of body (carapace length) and head size (head length). For most vertebrates, the presence of either negative allometry or isometry in head dimensions relative to body size is nearly universal. To my knowledge *S. minor* may be one of the only vertebrate taxa that collectively displays positive allometry in head length, width, and depth relative to body size. Bite-force generation did not scale to body or head size, or beak measures, in the proportions predicted from isometric growth. Bite-force generation scaled with negative allometry relative to lower beak depth and symphysis length. These results indicate the development of a more robust mandible that resists bending and shear
stresses, and facilitates force transmission. Nevertheless, bite-force generation scaled with positive allometry relative to all other morphological measurements. These data indicated that ontogenetic modifications to the lever mechanics of the jaw system, and/or changes in the size (i.e. mass) and/or physiology (e.g., fiber lengths, degree of pennation) of the jaw adductor musculature will have more explanatory power for bite-force generation than external measures.

Muscular dissections indicated that bite force in *S. minor* are almost entirely generated by the pennate subdivisions of the M. add. mand. externus. The theoretical bite-force model based on muscle-force generation and lever mechanics accurately predicted both the individual and the ontogenetic scaling of bite forces. Consequently, the underlying morphology of the musculoskeletal system was a good predictor of observed performance and was examined to determine which parameters explain the disproportionate increase in bite-force generation. Changes in the mechanical leverage of the feeding apparatus did not explain the observed phenomenon. Instead, the combined affects of increasing muscle masses and changing the muscle architecture of the pennate muscle bodies, resulted in an increase in total physiological cross-sectional area (*P*<sub>CSA</sub>) of the jaw adductor muscles that was proportional to change in bite forces. These results indicated that the disproportionate increase in bite-force generation relative to skull length found in *S. minor* was explained by allometric changes in muscle size and architecture that collectively act to elevate the *P*<sub>CSA</sub> and muscle force. In addition, dietary data indicated that the positive allometry in bite-force generation observed in *S. minor* is tightly linked to the incorporation of exponentially larger snails into the diet and positive allometry of the forces required to fracture the largest dietary items. These forces were found to be greater than the observed and theoretical bite forces, which suggested that fatigue failure resulting from multiple bite-force loadings may allow *S. minor* to fracture snails at lower compressive forces and access large snails that are apparently outside the range of their bite-force capacity.

Age determination via skeletochronology indicates that cranial expansion does not show a sudden divergence from growth of the carapace, instead cranial hypertrophy in these animals is achieved by a continuous pattern of enlargement that is present at all stages of development. Moreover, age-based growth patterns for bite-force generation tend to fit a logistic growth curve and tend to reflect a close relationship with the forces required to fracture snails found in the diet. These results provide additional empirical support for the close relationship between the performance of the feeding apparatus and dietary ecology, and suggest that the development of
cranial hypertrophy and elevated bite-force generation may be a plastic response to increased mechanical stress incurred during feeding.

For this work, I have identified the morphological traits that are important for an ontogenetic change in performance and established why this performance trait is ecologically important to *S. minor*. Because ecological performance is directly linked to morphological design and ultimately determines the fitness of individuals, understanding how variation in morphological traits relates to differences in performance provide insights into the mechanistic and functional issues that underlie the evolution of phenotypic traits. Consequently, similar studies to the one presented here are imperative for developing a more comprehensive understanding of ecology and evolution in gnathostome vertebrates.
APPENDIX

ANIMAL SUBJECTS APPROVAL LETTER

AND

SIGNIFICANT CHANGES FORM WITH APPROVAL NOTIFICATION
FLORIDA STATE UNIVERSITY
ANIMAL CARE AND USE COMMITTEE
PROTOCOL REVIEW FORM

Principal Investigator:  Gregory M. Erickson, Ph. D.  Date:  May 26, 2006
E-Mail Address:  gerickson@bio.fsu.edu  Telephone:  645-4981
Campus Address:  316 Conradi Building  Mail Code:  1100
Department:  Biological Science  ACUC Protocol #:  0011
Proposal Title:  Form, Function, Development, and Evolution of the Vertebrate Musculo-Skeletal system

X THIS PROTOCOL IS SUBMITTED AS A TRI-ANNUAL RENEWAL.

There ARE current personnel changes. Please list all personnel that work with animals who have been added to, or deleted from this project.

The FSU ACUC acknowledges that replication of previous research may be an essential element in scientific inquiry. In planning this experiment, I have reviewed the relevant literature (e.g., database search, consultation with colleagues, other). Based upon the available resources, I certify that the work described in this protocol does not unnecessarily duplicate previous work.

P.I. Signature:  
Date:  May 26, 2006

Your signature acknowledges your responsibility for the contents of this questionnaire and the conduct of any animal use that may be approved by the Committee, by yourself or your staff. Any significant change in procedure, animal use or personnel must be approved by the ACUC prior to implementation. All animal procedures proposed in grant submissions must be included in an ACUC Animal Care and Use Protocol and be reviewed and approved by the ACUC. Failure to do so may result in suspension of approval to perform animal research at FSU as well as suspension of funding.

This Questionnaire must contain all animal use information necessary for ACUC approval. Please exclude any confidential information from this questionnaire (e.g. proprietary information, potential trade secrets, and patentable material) as the document is considered a public record and is available to outside parties by request under the Freedom of Information Act. If confidential material must be included, please mark it "Confidential" and contact the secretary, ACUC, for further instructions.

ACUC Approved:  06/28/2006  Next Review Date:  05/01/2009
Florida State University  
Animal Care and Use Committee [ACUC]  
SIGNIFICANT CHANGE FORM

<table>
<thead>
<tr>
<th>Principal Investigator:</th>
<th>Dr. Gregory M. Erickson</th>
<th>Date:</th>
<th>March 1, 2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-Mail Address:</td>
<td><a href="mailto:gerickson@bio.fsu.edu">gerickson@bio.fsu.edu</a></td>
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<td>645-4991</td>
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<td>1100</td>
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<tr>
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<td>Form, Function, Development and Evolution of the Vertebrate Musculo-Skeletal System</td>
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I wish to make significant changes in this protocol as described below or in the attached and I request ACUC review. (Identify attachment only by protocol # and date).

- Animal Increase
- Addition of Species
- **X** Procedure Modification and/or Addition

**Personnel Changes**
Please list all personnel that work with animals who have been added to or deleted from this project.

ADD:  
DELETE:

**NOTE 1:** It is the responsibility of the Principal Investigator to ensure that all personnel who have animal contact be enrolled in the FSU Medical Monitoring Program before they begin working with the animals. This requirement must be addressed or the ACUC may either not approve the protocol or may suspend approval of the protocol. Enrolling in the program can be accomplished by filing out the Medical Monitoring for Vertebrate Animal Users Form (http://www.safety.fsu.edu/forms.html) or contact your Departmental Representative for the forms and assistance or contact Environmental Health and Safety at 644-9117.

**NOTE 2:** All Personnel must have completed the ACUC required training prior to or within 14-days from beginning work with animals. This requirement must be addressed or the ACUC may either not approve the protocol or may suspend approval of the protocol. Please contact Laboratory Animal Resources, 101 BRF, or phone at 644-4262 for assistance in scheduling training.

Principal Investigator signature:
(This form is available electronically in either Word or WordPerfect. Please contact the ACUC Secretary to receive it electronically.)

ACUC APPROVED: June 14, 2007  
Revised: 06/2004
REFERENCES


BIOGRAPHICAL SKETCH

Personal:
Born 10 May, 1983; St. Louis, Missouri

Educational Background:
   Honors Thesis Title: Distribution patterns of epibionts on the carapace of nesting
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Research Experience:
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   University of Florida, Gainesville, FL ---- Collaborating Researcher with Queensland
   Environmental Protection Agency, Colin J. Limpus, Queensland, Australia. October 2005
   to June 2006.
Student Research Internship. Caretta Research Project, Directors: Kristina L. Williams and
Undergraduate Research Volunteer. Harvey B. Lillywhite, Department of Zoology, University of
   Florida, Gainesville, FL. (September 2004 – August 2005)
Undergraduate Research Volunteer. Archie Carr Center for Sea Turtle Research, Director: Karen
   A. Bjorndal, University of Florida, Gainesville, FL. (September 2003 – April 2005)
Undergraduate Research Volunteer. Louis J. Guillette Jr., Department of Zoology, University of
Student Research Assistant. Molecular Epidemiology and Fungus Testing Laboratory, Associate
   Professor of Medicine: Daniel C. Diekema, University of Iowa, Iowa City, IA. (January
Undergraduate Research Volunteer. Philip C. Darby, Department of Biology, University of West

Teaching Experience:
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Graduate Teaching Assistantship. Comparative Vertebrate Anatomy (ZOO3713C), Instructor:
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Graduate Teaching Assistantship, Animal Diversity (BIO2011L), Instructor: Gavin J.P. Naylor. Department of Biological Sciences, Florida State University, Tallahassee, FL. (Fall 2007)
Graduate Teaching Assistantship, Herpetology: Biology of Lower Vertebrates (ZOO4343C), Instructor: Gregory M. Erickson. Department of Biological Sciences, Florida State University, Tallahassee, FL (Fall 2006, 2008)

Conference Presentations:


Pfaller, J.B., and M.G. Frick. January 2008. Sea Turtle Epibiosis: A case where it’s okay to have crabs. Sea Turtle Epibiont Workshop at the 28th Symposium on Sea Turtle Biology and Conservation, Loreto, Baja California, Mexico. (Oral Presentation)


Publications:


Pfaller, J. B., K. A. Bjorndal, K. J. Reich, K. L. Williams, and M. G. Frick. 2006. Distribution patterns of epibionts on the carapace of loggerhead turtles, Caretta caretta. Journal of the
