5-2018

The Functional Significance of Structural Novelty in the Locomotor Apparatus of Turtles

Christopher James Mayerl
Clemson University, matrim3@gmail.com

Follow this and additional works at: https://tigerprints.clemson.edu/all_dissertations

Recommended Citation
Mayerl, Christopher James, "The Functional Significance of Structural Novelty in the Locomotor Apparatus of Turtles" (2018). All Dissertations. 2121.
https://tigerprints.clemson.edu/all_dissertations/2121

This Dissertation is brought to you for free and open access by the Dissertations at TigerPrints. It has been accepted for inclusion in All Dissertations by an authorized administrator of TigerPrints. For more information, please contact kokeefe@clemson.edu.
THE FUNCTIONAL SIGNIFICANCE OF STRUCTURAL NOVELTY IN THE LOCOMOTOR APPARATUS OF TURTLES

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy Biological Sciences

by
Christopher James Mayerl
May 2018

Accepted by:
Dr. Richard W. Blob, Committee Chair
Dr. Michael W. Sears
Dr. Margaret B. Ptacek
Dr. Gabriel Rivera
ABSTRACT

The relationship between form and function can have profound impacts on the evolution and ecology of a lineage. Because of this relationship, variation in the morphology of a lineage has often been linked to adaptive radiations. However, form-function relationships are not linear, and variation in morphology does not necessarily predict variation in function due to the pervasive presence of mechanical equivalence in physiological systems. This possibility is often investigated through the lens of biomechanics, which uses physical principles to create a framework for comparing different systems with similar mechanical behaviors. Turtles represent an excellent system for studying how variation in structure might impact function. All extant turtles have descended from an aquatic common ancestor, and can be differentiated into two clades: cryptodires and pleurodires. These two clades can be distinguished by their pelvic girdle morphology. Cryptodires have an ancestral pelvic girdle morphology where the pelvis articulates with the sacral vertebrae at a joint, whereas pleurodires possess a derived morphology in which the pelvic girdle has been fused to the shell.

My dissertation investigates the functional role of pelvic girdle fusion in pleurodire turtles by studying functional differences in the musculoskeletal system between pleurodires and cryptodires, and then by investigating how these functional differences might impact performance in water and on land. First, I evaluate differences in girdle movements between cryptodire and pleurodire turtles using X-ray Reconstruction of Moving Morphology. Next, I examine how pelvic girdle fusion impacts muscle function and use during walking and swimming. Third, I studied the
potential for this novel structure to influence swimming performance. Finally, I compare the bone loading regimes of pleurodires with cryptodires during terrestrial locomotion. Data from these studies provide insight into the functional importance of novel structures and how they can impact the ecological and evolutionary history of lineages.
DEDICATION

This dissertation is dedicated to my family. My parents (James and Laura) have supported me in all that I’ve ever pursued, and even tried to read my papers! They always encouraged my curiosity, and I truly wouldn’t be here without them. They’ve also been taking care of my pet tortoises for the past twelve years while I gallivanted off to college. My brother Steven is with me in all that I do, and even if he doesn’t know it, he’s one of the biggest reasons why I strive to be as good as I can be. His kind heart and giving nature make sure that I always remember the important things in life. My dog Emris has brightened my life for the past two and a half years, and he makes sure that I always take time to relax and play, even on the most stressful day. Finally, this dissertation is dedicated to my fiancé, Alysia Arellanez. Alysia is the best thing that has ever happened to me, and her unwavering support and love keeps me motivated and inspired. This dissertation truly wouldn’t have been complete without her, or the rest of my family’s support and love, and I’ll forever be grateful for all that they have given me.
ACKNOWLEDGMENTS

This dissertation may have my name on it, but it would not have been possible without the many people who have supported me throughout my education. First, I’d like to thank my dissertation committee: Drs. Margaret Ptacek, Michael Sears, and Gabriel Rivera, who were always available to offer guidance on advice from topics as wide ranging as statistics to life advice. I’d like to especially thank the chair of my committee, Dr. Rick Blob, who has been an unwavering source of support throughout my time at Clemson. Rick has always been there to guide my professional development, but more importantly he’s also helped me to grow as a person and a scientist.

I’d also like to thank my friends and family for their support. My colleagues in science have inspired me daily, for which I am forever grateful: Eric Riddell, Achyuthan Srikanthan, Sandy Kawano, Kylie Smith, and Kelly Diamond. From a young age my parents told me that I should just follow my passion in choosing my career, and they have been there to support me through all my (numerous) struggles. My brother keeps me motivated when things get rough, and I always strive to be the best that I can be for him. Emris keeps me grounded, and manages to put a smile on my face, no matter how hard the day. Finally, I’d like to thank my best friend, my hero, and my partner for always believing in me (even when I didn’t), always fighting for me, and always supporting me, Alysia Arellanez.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE ...............................................................</td>
</tr>
<tr>
<td>ABSTRACT .................................................................</td>
</tr>
<tr>
<td>DEDICATION ....................................................................</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS ..........................................................</td>
</tr>
<tr>
<td>LIST OF TABLES ..............................................................</td>
</tr>
<tr>
<td>LIST OF FIGURES ...........................................................</td>
</tr>
<tr>
<td>CHAPTER</td>
</tr>
<tr>
<td>I. INTRODUCTION ................................................................</td>
</tr>
<tr>
<td>References .....................................................................</td>
</tr>
<tr>
<td>II. PELVIC GIRDLE MOBILITY OF CRYPTODIRE AND PLEURODIRE TURTLES DURING WALKING AND SWIMMING ................................................</td>
</tr>
<tr>
<td>Abstract .......................................................................</td>
</tr>
<tr>
<td>Introduction ..................................................................</td>
</tr>
<tr>
<td>Materials and Methods ...............................................</td>
</tr>
<tr>
<td>Results .................................................................</td>
</tr>
<tr>
<td>Discussion ...................................................................</td>
</tr>
<tr>
<td>Acknowledgements .....................................................</td>
</tr>
<tr>
<td>References ..................................................................</td>
</tr>
<tr>
<td>III. HINDLIMB MUSCLE FUNCTION IN TURTLES: IS NOVEL SKELETAL DESIGN CORRELATED WITH NOVEL MUSCLE FUNCTION? .........................</td>
</tr>
<tr>
<td>Abstract ....................................................................</td>
</tr>
<tr>
<td>Introduction ..................................................................</td>
</tr>
<tr>
<td>Materials and Methods ................................................</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Mean (± SE) pelvic girdle rotations (deg) for cryptodire (<em>Pseudemys concinna</em>) and pleurodire (<em>Emydura subglobosa</em>) turtles during walking and swimming, with mixed effects model significance (p-value) and effect size ($\Omega^2$).</td>
</tr>
<tr>
<td>2.2</td>
<td>Major and minor axes of the elliptical sliding motions (in mm ± SE) of the cryptodire pubis and ischium relative to the plastron during walking and swimming.</td>
</tr>
<tr>
<td>2.3</td>
<td>Mean femoral rotation (± SE) in each direction, relative to the neutral reference pose for cryptodire (<em>Pseudemys concinna</em>) and pleurodire (<em>Emydura subglobosa</em>) turtles during swimming and walking with discriminant analysis loadings.</td>
</tr>
<tr>
<td>3.1</td>
<td>Differences between cryptodire and pleurodire turtles in normalized muscle physiological cross sectional areas (PCSA) and size-normalized moment arm (MA) for directions of hindlimb motion.</td>
</tr>
<tr>
<td>3.2</td>
<td>Muscle activity patterns during locomotion while swimming and walking in cryptodire and pleurodire turtles.</td>
</tr>
<tr>
<td>4.1</td>
<td>Comparison of standardized limb lengths and areas (measurements divided by carapace length and carapace length squared, respectively) between <em>E. subglobosa</em> (pleurodire, N = 6) and <em>C. picta</em> (cryptodire, N = 7) turtles.</td>
</tr>
</tbody>
</table>
List of Tables (Continued)

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Peak axial (εaxial), principal tensile (εt), principal compressive (εc) and shear strains from <em>P. niger</em> and <em>P. concinna</em> femur during walking.</td>
<td>171</td>
</tr>
<tr>
<td>5.2</td>
<td>Principal tensile (εt), principal compressive, (εc) and shear strains for an individual <em>P. niger</em> with irregular loading regime.</td>
<td>172</td>
</tr>
<tr>
<td>5.1</td>
<td>Mechanical properties, estimated peak strains and mean safety factors for <em>P. niger</em> and <em>P. concinna</em>.</td>
<td>173</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>2.1</td>
<td>X-ray images of walking turtle in lateral (A) and ventral (B) views.</td>
<td>47</td>
</tr>
<tr>
<td>2.2</td>
<td>Joint coordinate systems (JCSs) used in this study from the pleurodire turtle, <em>Emydura subglobosa</em>.</td>
<td>48</td>
</tr>
<tr>
<td>2.3</td>
<td>Pelvic girdle rotations in cryptodire (<em>Pseudemys concinna</em>) and pleurodire (<em>Emydura subglobosa</em>) turtles during walking and swimming.</td>
<td>49</td>
</tr>
<tr>
<td>2.4</td>
<td>Cryptodire pelvic girdle translations during walking.</td>
<td>51</td>
</tr>
<tr>
<td>2.5</td>
<td>Multiple regression results illustrating the effect of increasing femoral excursion and stride frequency on pelvic rotation during walking (A), and swimming (B) in a cryptodire turtle, <em>Pseudemys concinna</em>.</td>
<td>52</td>
</tr>
<tr>
<td>2.6</td>
<td>Canonical discriminant function analysis of femoral movements of cryptodire (<em>Pseudemys concinna</em>) and pleurodire (<em>Emydura subglobosa</em>) turtles.</td>
<td>53</td>
</tr>
<tr>
<td>2.7</td>
<td>Mean femoral long axis rotation (LAR) excursions of cryptodire (<em>Pseudemys concinna</em>) and pleurodire (<em>Emydura subglobosa</em>) turtles during walking and swimming.</td>
<td>54</td>
</tr>
<tr>
<td>3.1</td>
<td>Isolated hindlimb muscles of turtles that have experienced a change in their location of origin associated with pelvic girdle fusion in the pleurodire lineage.</td>
<td>88</td>
</tr>
</tbody>
</table>
List of Figures (Continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>90</td>
</tr>
<tr>
<td>3.3</td>
<td>91</td>
</tr>
<tr>
<td>3.4</td>
<td>92</td>
</tr>
<tr>
<td>3.5</td>
<td>93</td>
</tr>
<tr>
<td>3.6</td>
<td>95</td>
</tr>
<tr>
<td>3.7</td>
<td>97</td>
</tr>
</tbody>
</table>

3.2 Representative still images from lateral (A) and ventral (B) views showing landmarks digitized for kinematic analysis.

3.3 Size normalized moment arms in ab/adduction for the five focal hindlimb muscles for each species (grey circle = cryptodire, *Trachemys scripta* (N = 6 individuals); black diamond = pleurodire, *Emydura subglobosa* (N = 6 individuals)).

3.4 Hindlimb muscle use while swimming (A) and walking (B) in cryptodire (*T. scripta*) (black) and pleurodire (*E. subglobosa*) (blue) turtles.

3.5 Canonical discriminant function analysis of hindlimb muscle activity patterns in cryptodire (*T. scripta*) and pleurodire (*E. subglobosa*) turtles.

3.6 Mean hindlimb kinematics of cryptodire (*T. scripta*, black) and pleurodire (*E. subglobosa*, blue) turtles while swimming (left, cryptodire: N = 5 individuals, 84 cycles; pleurodire: N = 6 individuals, 149 cycles) and walking (right, cryptodire: N = 5 individuals, 88 cycles; pleurodire: N = 6 individuals, 116 cycles).

3.7 Canonical discriminant function analysis of hindlimb kinematics in cryptodire (*T. scripta*) and pleurodire (*E. subglobosa*) turtles.
List of Figures (Continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Anatomical landmarks used to track limb and body movements for evaluation of stability and turning performance during swimming.</td>
<td>131</td>
</tr>
<tr>
<td>4.2</td>
<td>Violin plots comparing the stability (in excursion) of <em>E. subglobosa</em> (pleurodire) and <em>C. picta</em> (cryptodire) during linear swimming in (A) heave, (B) pitch, (C) sideslip, and (D) yaw.</td>
<td>132</td>
</tr>
<tr>
<td>4.3</td>
<td>Violin plots comparing turning performance between <em>E. subglobosa</em> (pleurodire) and <em>C. picta</em> (cryptodire).</td>
<td>133</td>
</tr>
<tr>
<td>4.4</td>
<td>Multivariate kinematic results during linear swimming (A) and turning (B). Green: <em>E. subglobosa</em> (pleurodire); blue: <em>C. picta</em> (cryptodire).</td>
<td>134</td>
</tr>
<tr>
<td>4.5</td>
<td>Violin plots comparing mean limb velocity for <em>E. subglobosa</em> (green) and <em>C. picta</em> (blue).</td>
<td>135</td>
</tr>
<tr>
<td>5.1</td>
<td>Representative simultaneous strain recordings from three gauge locations on the femur of <em>P. niger</em> during three consecutive steps.</td>
<td>165</td>
</tr>
<tr>
<td>5.2</td>
<td>Representative strain recordings (simultaneous) from three gauge locations on the femur of <em>P. concinna</em> during three consecutive walking steps.</td>
<td>167</td>
</tr>
<tr>
<td>5.3</td>
<td>The relationship between peak strain magnitude (in με) and peak strain rate (in με s⁻¹) for three <em>P. niger</em> turtles (N = 69 steps).</td>
<td>168</td>
</tr>
<tr>
<td>5.4</td>
<td>Graphical comparisons of cross-sectional planar strain analyses of femoral strain distributions calculated at five time increments (as a percentage of stance) during representative walking for two <em>P. concinna</em> (Pc05, Pc03, Butcher et al., 2008) and two <em>P. niger</em> (Pn03, Pn04).</td>
<td>169</td>
</tr>
</tbody>
</table>
CHAPTER ONE
INTRODUCTION

Studies on the relationship between the form and function of organisms have a long history in biology (Cuvier, 1798; Lauder, 1990; Taylor and Thomas, 2014). These studies have often been rooted in the idea that organisms can only evolve by adhering to the unassailable laws of physics (Maynard Smith et al., 1985), resulting in variation in the form, or structure of organisms potentially influencing their performance which, ultimately, can impact their fitness (Arnold, 1983; Grant and Grant, 1996; Kingsolver and Huey, 2003). In particular, traits that represent structural innovations have the potential to be a major force shaping evolutionary patterns of diversification (Wainwright and Price, 2016).

Innovations are novel traits (or a combination of traits) that result in changes in performance in the lineage that has evolved them (Liem, 1973; Losos, 2010; Wainwright and Price, 2016). Such traits can influence the potential for phenotypic variation in a lineage by changing the range of morphospace occupied by the lineage, or they can enable a lineage to move into new regions of an adaptive landscape, within which new peaks might be reached (Wainwright, 2007). Innovations in structural design can serve as a causative agent to spur bouts of morphological, functional, and ecological diversification, and have served as key features in adaptive radiations (Losos, 2010). However, innovations may have little effect on diversification, or might even constrain it and facilitate increased extinction rates in the lineages that evolved them (Wainwright, 2007; Mcgee et al., 2015).
Innovations that enhance diversification are often referred to as ‘key innovations’, such as the evolution of wings in insects and birds (Middleton and Gatesy, 2000; Nicholson et al., 2014), and the evolution of jaw protrusion and spiny fins in bony fishes (Acanthomorphs, Wainwright and Longo, 2017). Innovations that constrain diversification often lead to the specialization of a lineage such as the evolution of toe pads in geckos (Higham et al., 2015), and modified pharyngeal jaws in fishes (Mcgee et al., 2015). In order to evaluate the potential of a novel phenotypic trait to have served as a key innovation, as a constraint to diversification, or as a structure that does not impact diversification we must first test the relationship between form and function of the traits in question.

**Relationships between form and function**

The relationship between form and function in organisms is not always straightforward; not all morphological variation leads to equivalent functional consequences (Koehl, 1996; Wainwright et al., 2005; Stayton, 2006; Taylor and Thomas, 2014). Moreover, multiple morphological configurations can result in similar functional outputs. This capacity has been termed mechanical equivalence, or ‘many-to-one mapping’ (Anderson and Patek, 2015; Wainwright, 2007). Many-to-one mapping is thought to be a common property of many physiological systems, and can significantly impact the diversity and ecology of a lineage (Alfaro et al., 2005). Because of this possibility, any proposed ideas about ecological or evolutionary consequences of
morphological innovations should be founded on investigations of the function of the innovation itself.

In addition, investigations of function must also consider the potential for physiological and biomechanical tradeoffs, because modifications that improve performance in one behavior may come at a cost to performance in others (Ghalambor et al., 2004; Toro et al., 2004). Tradeoffs in physiological and biomechanical performance are ubiquitous in functional systems. For example, there are tradeoffs between the ability to withstand a large range of temperatures and having higher performance in a small range (Huey and Slatkin, 1976), as well as tradeoffs in individual muscles between burst and sustained performance (Vanhooydonck et al., 2014). Tradeoffs also extend into feeding and locomotor performance. For example, fishes that bite to acquire food are worse at producing suction (Wainwright et al., 2004), and there is a tradeoff between feeding performance and growth (Schluter, 1995). In the locomotor systems of animals, tradeoffs are ubiquitous both between environments and between behaviors within a given environment. Animals that are excellent swimmers usually exhibit poor terrestrial locomotor performance and vice versa (Shine and Shetty, 2001; Isaac and Gregory, 2007). Furthermore, within a given environment, animals that are highly stable usually exhibit decreased turning performance (Vogel, 1994; Walker and Westneat, 2000). These trade-offs in organismal performance can serve as powerful constraints in evolution (Ghalambor et al., 2004). The presence of tradeoffs in most animal systems, as well as the potential for many-to-one mapping in these systems, means that the relationship
between a structural innovation and its function must be tested before inferences about its role in an animal’s ecology and evolutionary history can be drawn.

Form-function relationships are often tested through the context of biomechanics, which uses physical principles to create a framework for comparing different biological systems with similar mechanical behaviors (Anderson et al., 2016). Examples of such approaches include comparing relationships between skeletal morphology and locomotor behavior (Baier et al., 2013; Mayerl et al., 2016), evaluating the consequences of different linkage systems for muscle power output (Wainwright et al., 2004; Anderson et al., 2014; Anderson and Patek, 2015; Olsen and Westneat, 2016;), or determining the impact of differences in mechanical advantage on muscle function (Carrier, 1996; Mayerl et al., 2017; Rivera and Blob, 2013; Stubbs et al., 2013). These approaches allow the functional impact of structural innovations to be tested, providing a gateway for evaluating the potential for functional tradeoffs and consequences of form-function relationships for diversification.

_Turtles as a model system in studies of form-function relationships_

Turtles provide a rich system for studying how structural innovations have impacted form-function relationships, particularly in the context of locomotor performance. As a result of the vertebral column fusing with the dorsal part of the shell (the carapace), all thrust for locomotion is produced by the limbs, making comparisons in performance particularly tractable (Pace et al., 2001). Although the general bauplan of turtles has remained fairly constant (e.g. having a bony shell with a fused vertebral
column) for over 200 million years, there is extensive variation within turtles in both their morphology and ecology. Turtles live in diverse habitats that span a range from fully terrestrial to fully aquatic (Bonin et al., 2006). Even among turtle species that inhabit the same environment, there are differences in resource use and habitat partitioning (Cann, 1998; Bonin et al., 2006). Across such habitats there is also extensive variation in turtle morphology. Terrestrial turtles often have a domed carapace, whereas aquatic turtles have a flattened, streamlined carapace that reduces the hydrodynamic demands of water (Bonin et al., 2006; Vogel, 2013), especially when living in environments with flowing water (Rivera, 2008).

The behavioral and ecological variation in turtles has led to them serving as a model system for studies on the impact of different environments and morphologies on locomotor performance. Swimming turtles employ two general propulsive modes, rowing and flapping. Rowing is a drag-based method of locomotion and involves primarily anteroposterior movements of contralateral limbs, whereas flapping is lift based, and in turtles is accomplished primarily with the forelimbs moving in a dorsoventral direction. Freshwater turtles primarily employ rowing and sea turtles use flapping to move through their respective environments. Studies on these behaviors have shown that they can be achieved via anatomical changes to the musculoskeletal system as well as changes in neuromotor control, and that these different behaviors result in different levels of performance (Rivera and Blob, 2010; Rivera et al., 2011a). Due to the amphibious habits of many taxa, turtles have also served as a model system for investigations of how animals deal with the conflicting demands of moving in water versus moving over land.
In addition, because of their distinctive morphology with the vertebral column fused to the shell, they also serve as a model for measuring swimming performance in rigid bodied animals (Rivera et al., 2006; Rivera et al., 2011a). Such studies, however, have typically focused on only one of the two major lineages of turtles – the cryptodire lineage that is common across the Northern Hemisphere in North America, Europe, and Asia. The other major lineage of turtles, the pleurodires, is distinguished from cryptodires by several features, including traits of the locomotor system that could provide additional avenues to assess the impact of structural innovations on functional and evolutionary diversification.

Pleurodires and cryptodires are thought to have separated approximately 145 million years ago, and the distinction of these lineages is supported by a variety of molecular and morphological analyses (Gaffney, 1975; Gaffney et al., 2007; Guillen et al., 2012; Crawford et al., 2014). The ancestor of all extant turtles is thought to have been primarily aquatic, and although pleurodire turtles have not radiated onto land in their evolutionary history, cryptodires have done so multiple times independently (e.g. the tortoises, box turtles, and Asian box turtles) (Joyce and Gauthier, 2004; Joyce et al., 2013). One morphological distinction between the two groups is that cryptodires possess an ancestral pelvic girdle morphology that is not fused to the shell, whereas pleurodires exhibit a structural novelty in that their pelvis has been fused to the shell (Walker, 1973). Despite this feature serving as a distinctive trait defining the two lineages, the functional consequences of pelvic girdle fusion in pleurodire turtles have not been evaluated. My dissertation examines the functional consequences of this structural innovation for
locomotor performance, with the goal of evaluating its potential role in explaining the differing ecological distributions of these lineages.

Fusion of the pelvic girdle to the shell could have important consequences for the locomotor performance and diversification of pleurodire turtles. In many animals, rotations of the axial skeleton and pelvic girdle increase stride length and decrease the overall twisting loads on the femur, thereby improving locomotor performance (Delvolvé et al., 1997; Reilly and Delancey, 1997; Russell and Bels, 2001; Butcher and Blob, 2008; Arnold et al., 2014). As all turtles have an inflexible axial skeleton, and thus rely solely on limb movements to provide thrust for locomotion (Zug, 1971; Pace et al., 2001; Blob et al., 2008), pelvic girdle rotations provide the only mechanism for increasing stride length beyond movement of the limbs. Immobilization of the pelvic girdle through its fusion to the shell might decrease extraneous lateral movements, and possibly contribute to an increase in stability. Such stabilization could be especially important during aquatic locomotion, due to the energetic costs of correcting movements against a medium like water that imposes high levels of drag. However, femoral loads might also differ between turtle lineages with differences in pelvic girdle morphology, as an immobile pelvis cannot move in conjunction with the stride to decrease twisting loads on the femur (Butcher et al., 2008). This would be especially important during terrestrial locomotion, when strains are at their peak as the animal must support its body weight against gravity. If such contrasts in performance are present in turtle lineages with differing pelvic girdle morphology, they might be a factor contributing to the primarily aquatic distributions of
pleurodires, in contrast to the multiple successful invasions of terrestrial habitats by their cryptodire counterparts.

The fusion of the pelvis to the plastron in pleurodires has also resulted in shifts in muscle attachments in this taxon (Walker, 1973). Two major factors commonly viewed as contributing to locomotor diversity are variation in the morphology of locomotor structures, and variation in the activation patterns of the muscles that power locomotion (Lauder and Reilly, 1996). A prominent hypothesis that emerged from early comparative studies was that novel locomotor behaviors (i.e., patterns of movements) might evolve primarily through structural changes (Dial et al., 1991), rather than changes in muscle activity – a prediction termed the “neuromotor conservation hypothesis” (Smith, 1994). However, some research has found mixed results that do not completely support this prediction (Rivera et al., 2011b; Rivera and Blob, 2013), and the pervasiveness of neuromotor conservation remains controversial. Freshwater turtles are thus an advantageous system to test neuromotor conservation, as cryptodires and pleurodires exhibit relatively similar locomotor strategies (antero-posterior rowing), despite changes in musculature attachments at the hindlimb. These shifts in muscle attachments may have resulted in pleurodires either using their muscles differently than cryptodires, or may contribute to differences in kinematics between the two taxa.

This work is the first to examine the biomechanics and kinematics of locomotion in pleurodire turtles, the sister group to all other turtles. Collecting these data and making comparisons with cryptodires will provide insight into the evolution of locomotor performance in one of the most distinctive of all extant vertebrate groups. Understanding
the relationship between the physical characteristics of organisms and their interactions with the environment is particularly important in understanding the functional consequences of structural innovations and the role that these innovations can serve to either constrain or facilitate evolutionary diversification.

To investigate the functional role of the novel pelvic girdle fusion found in pleurodire turtles, I performed a series of experiments that integrated measurements of musculoskeletal function and organismal performance. Chapter 2 studies differences in pelvic girdle motion between pleurodire and cryptodire turtles during locomotion on land and in water. This work was published in the Journal of Experimental Biology, and was featured as the cover image in the issue it was published. Chapter 3 builds upon Chapter 2 by investigating the consequences of pelvic girdle fusion for the musculoskeletal system of the hindlimb in pleurodire turtles. I combined measurements of muscle lever mechanics with electromyography to test for differences in muscle use between water and land in cryptodire and pleurodire turtles. This work has also been published in the Journal of Experimental Biology. These data served as a foundation for Chapter 4, in which I studied the potential for pelvic girdle fusion to contribute to increased swimming performance in pleurodires when compared to their cryptodire relatives. I found that pleurodires had both higher stability, and higher turning performance than cryptodires, two metrics of performance that usually involve tradeoffs in performance. Finally, Chapter 5 details my research on the potential that pelvic girdle fusion has inhibited pleurodires from diversifying onto land by resulting in increased femoral torsion when walking. Collectively, these studies use an integrative approach to investigate the idea
that structural innovations in the musculoskeletal systems of animals can lead to functional consequences for their performance in different environments.
REFERENCES


**Pace, C. M., Blob, R. W. and Westneat, M. W.** (2001). Comparative kinematics of the forelimb during swimming in red-eared slider (*Trachemys scripta*) and spiny


Academic Press.


ABSTRACT

Movements of the pelvic girdle facilitate terrestrial locomotor performance in a wide range of vertebrates by increasing hind limb excursion and stride length. The extent to which pelvic movements might contribute to limb excursion in turtles is unclear, because the bony shell surrounding the body presents a major obstacle to their visualization. In cryptodires, one of the two major lineages of turtles, pelvic anatomy indicates the potential for rotation inside the shell. However, in pleurodires, the other major lineage of turtles, the pelvis shows a derived fusion to the shell, likely preventing pelvic motion. In addition, most turtles use their hind limbs for propulsion during swimming as well as
walking, and the different locomotor demands between water and land could lead to differences in the contributions of pelvic rotation to limb excursion in each habitat. To test these possibilities, we used X-ray Reconstruction of Moving Morphology (XROMM) to compare pelvic mobility and femoral motion during walking and swimming between representative species of cryptodire (*Pseudemys concinna*) and pleurodire (*Emydura subglobosa*) turtles. We found that the pelvis yawed substantially in cryptodires during walking and, to a lesser extent, during swimming. These movements contributed to cryptodires having greater femoral protraction in both walking and swimming when compared to pleurodires, in which the pelvis was immobile. Though factors related to the origin of pelvic-shell fusion in pleurodires are debated, its implications for their locomotor function may contribute to the restriction of this group to primarily aquatic habits.

**INTRODUCTION**

Limb excursion is critical to the locomotor performance of a wide range of animals, contributing to stride length, speed, distance travelled and, ultimately, impacting survival through effects on functions such as resource acquisition and escape from predators (Irschick and Jayne, 1999; Calsbeek and Irschick 2007). One mechanism used by a variety of vertebrates to increase limb excursion (and stride length) during terrestrial locomotion is the incorporation of pelvic motion into the stride (Jenkins, 1971; Pridmore, 1992; Reilly and Delancey, 1997; Russell and Bels, 2001). Frequently evaluated via kinematic measurements of external markers filmed with standard light videos (though
see Gatesy, 1991; Nyakatura et al., 2014), pelvic rotation, particularly yaw, can increase the anteroposterior arc of hind limb excursion, thereby lengthening terrestrial strides.

Though locomotor pelvic movements are widespread across many groups of vertebrates, novel body plans that have evolved in some taxa might impede the extent to which such motion is an effective mechanism for lengthening strides. For example, in turtles the dorsal vertebrae and sacrum are fused to a bony shell that surrounds the body, including the limb girdles (Walker, 1973; Gilbert et al., 2001). Not only does such enclosure of the pelvis potentially limit its motion in turtles, but it also obscures visualization of potential pelvic movements. In an early study of turtle locomotor function that sought to overcome this technical challenge, Walker (Walker, 1971) collected dorsal-perspective, X-ray films of walking by the painted turtle (*Chrysemys picta*), a semi-aquatic species from the cryptodire lineage. These images did not show evidence of pelvic girdle movements (Walker, 1971). However, they were collected while animals were suspended in the field of view by a cloth sling, potentially influencing natural movement patterns. In addition, Walker’s later, landmark study of musculoskeletal anatomy in turtles (Walker, 1973) noted the possibility for motion between the pelvis and shell in cryptodires, as the ilium is connected to the sacrum via a sliding joint, and the pubis and ischium are suspended above the ventral portion of the shell via the puboischadic ligament. This anatomical evidence suggests the possibility that pelvic rotation or translation might yet contribute to hind limb excursion in some turtle taxa under certain conditions.
Across the diversity of turtles, many species make frequent use of both aquatic and terrestrial habitats (Ernst and Lovich, 2009). Compared to many other semiaquatic vertebrates, however, turtles are distinctive in that the fusion of their vertebrae to the shell means that they must propel themselves with their limbs, rather than their body axis, in water as well as on land (Pace et al., 2001; Rivera et al., 2006). As a result, pelvic girdle motion provides the only potential contribution to hind limb excursion during swimming and walking in turtles beyond the motions of the limbs themselves. However, without contact of the foot with solid ground, reduced substrate reaction forces acting on the hind limb during swimming (Blob et al., 2003; Butcher and Blob, 2008) might decrease any contribution of pelvic rotation or translation to limb excursion in water, relative to land.

Girdle structure is a major factor in the evolutionary diversification of turtles (Joyce et al., 2013a), and may carry functional consequences that distinguish the locomotor performance of clades (Renous et al., 2008). In contrast to cryptodires, in the other main clade of turtles, the pleurodires, the pelvis exhibits a derived, bony fusion to both the dorsal (carapace) and ventral (plastron) portions of the shell (Walker, 1973; Joyce et al. 2013b). Such fusion would be expected to preclude any pelvic motion relative to the shell, limiting hind limb excursion. Due to the potential differences in girdle movements between cryptodires and pleurodires, turtles represent an excellent system for examining the effects of pelvic girdle mobility in vertebrate locomotion. However, no quantitative locomotor kinematics have been measured for any pleurodire species, making it difficult to assess the impact of the structural novelty of their pelvis on locomotor function.
To evaluate the potential contributions of pelvic rotation to locomotor performance in turtles, and to assess its variation across habitats and taxa with different pelvic structures, we used marker based X-Ray Reconstruction of Moving Morphology (XROMM; Brainerd et al., 2010) to measure the movements of the pelvis and femur relative to the shell in representative species of cryptodire and pleurodire turtles during both walking and swimming. We predicted that the pelvis would rotate in cryptodires during both behaviors, with rotation being greater on land than in water. We also predicted that fusion to the shell in pleurodires would prevent pelvic movements relative to the shell both in water and on land. By measuring motion of the femur relative to the pelvis we also obtained new measurements of long-axis rotation of the femur in both swimming and walking that are difficult to obtain through other techniques (Kambic et al., 2014; Kambic et al., 2015), and which inform understanding of how limb function and skeletal loading change between environments (Butcher and Blob, 2008; Young and Blob, 2015). These findings may have bearing on differences in locomotor function and habitat distribution between the cryptodire and pleurodire clades.

**MATERIALS AND METHODS**

**Experimental subjects**

XROMM analyses were performed for both walking and swimming in three adult males (660-1200 g, 185 – 223 mm) of the cryptodire *Pseudemys concinna* (LeConte 1830), the river cooter, and two adult males (610-675 g, 171 – 184 mm) of the pleurodire *Emydura subglobosa* (Krefft 1880), the Jardine river turtle. *P. concinna* specimens were collected
with hoop traps from a spillway of Lake Hartwell, Pickens County, SC, USA (South Carolina Scientific Collection Permit #29-2014). *E subglobosa* were obtained from a commercial supplier (Turtles and Tortoises Inc., Brooksville, FL). Prior to data collection, turtles were separated by species and housed at Clemson University in plastic tub enclosures that were half-filled with water and fitted with dry areas for basking. To collect XROMM data, turtles were transported to Brown University and housed in similar enclosures for the days over which data were collected. Turtles were fed commercial reptile pellets daily. All procedures and animal care were approved by the Institutional Animal Care and Use Committees (IACUC) of Clemson University (protocol 2015 – 001) and Brown University (protocol 1105990018).

**Surgical procedures**

Prior to data collection, 1 mm radio-opaque tantalum bead markers (Bal-Tec, Los Angeles, CA) were implanted into the pelvis, femur, and shell of each individual turtle (3-5 markers per bone), using aseptic technique (Fig. 1). In both turtle species studied, the carapace and plastron are firmly connected together by a bony bridge, so the shell was treated as a single rigid body. Surgeries were conducted at Clemson University, following published protocols (Brainerd et al., 2010). To induce analgesia and a surgical plane of anesthesia, doses of 1 mg kg⁻¹ butorphenol, 90 mg kg⁻¹ ketamine, and 1 mg kg⁻¹ xylazine were injected into the muscles of the forelimbs. To expose the pelvis and femur, single incisions were made on the ventral and dorsal aspects of the proximal region of the left hind limb. Muscles were separated along fascial planes to expose surfaces of the bones (Butcher et al., 2008). At each site of marker implantation, a small ‘window’ of
periosteum was removed to expose the bone cortex by gently scraping with a periosteal elevator. Each marker then was implanted by hand-drilling a 1 mm diameter hole into the bone with a pin vise, and pressing the bead into the hole with the stick handle of a cotton-tipped applicator. Markers were located in each of the three fused, triradiate bones that comprise the pelvis (ilium, pubis, and ischium), maximizing the distance of markers in this element from each other. Femoral markers were placed in proximal and midshaft regions as well as the distal condyles. Incisions were sutured closed once all markers were implanted. Each turtle was then allowed to recover on land for 24 hours before being placed in an individual aquatic enclosure with a basking platform.

**Experimental data collection**

Turtles recovered from surgery for 1-2 weeks before collection of XROMM data. Turtles were filmed during walking and swimming using biplanar X-Ray video. At the beginning, middle, and end of each day, X-ray images of standard grid and calibration objects were taken at the settings used for experimental trials.

During walking, a hand-powered treadmill was used to stimulate locomotion while minimizing magnetic interference with images. Two X-ray generators (Imaging Systems and Service, Painesville, OH, USA) were positioned in dorsal and lateral views. X-ray settings were 100 mA for both views, with kVp between 68 (laterally) and 85 (dorsally). X-ray images were recorded using Phantom v10 high-speed cameras (Vision Research, Wayne, NJ, USA) at a 1760 x 1760 pixel resolution. Data were recorded at 100 frames per second with a 1/500 s shutter speed. Turtles were allowed to rest approximately 10 minutes between trials, and 10 – 15 total steps were collected per turtle.
Swimming trials were filmed in a 161×61 cm acrylic aquarium filled to a depth of no more than 10 cm to minimize the amount of water through which X-rays had to pass before reaching animals. Turtles were stimulated to swim by placing them at the opposite end of the tank from a dark shelter, toward which they swam when released. X-ray generators were positioned over the left and right sides of the tank, angled 45° to the plane of animal movement and 90° from each other. X-ray settings were 100 mA for both views, with kVp between 90 (left view) and 100 (right view). Images were recorded as above, with data recorded at 150 frames per second and 1/1000 s shutter speed. Turtles were allowed to rest approximately 10 minutes between trials, and 10 – 15 total strokes were collected per turtle.

Following data collection, turtles were sedated with an intramuscular injection of ketamine (30 mg kg\(^{-1}\)), and computed tomography (CT) scans were taken of each individual with an Animage Fidex veterinary scanner with an 8-15 cm field-of view and 0.2-0.3 mm isotropic voxels. These CT scans were used to generate polygonal mesh models of the shell, pelvis, and femur in OsiriX (v. 3.9.2 64 bit, Pixmeo Sari Geneva, Switzerland) and Geomagic Design X 64 (v. 2016 0.1, Geomagic, Inc. Triangle Park, NC, USA).

**Data processing**

The X-ray video and CT scan data collected and analyzed for this study are available from the X-ray Motion Analysis Portal (xmaportal.org). Following data collection, X-ray videos were processed using XMALab v. 1.2.12 (open source;
Standard grid images were used to correct for distortions in the video that are introduced by X-Ray image intensifiers (Brainerd et al., 2010). Calibration objects of known geometry (cubes with 64 radio-opaque markers) were used to calibrate the 3-D space (Brainerd et al., 2010). After tracking markers (3-5 per bone, Fig. 1), rigid body motions of the femur, pelvis, and shell were filtered with a Butterworth low-pass filter (10 Hz cutoff for walking and 15 Hz for swimming) and exported. Animations were constructed by applying these motions to polygonal mesh models of the bones in Autodesk Maya (2014, Autodesk Inc., San Rafael, CA, USA).

To describe the 3-D movements of the pelvis (Fig. 2A) and femur (Fig. 2B), we created anatomical coordinate systems (ACSs) for each of these elements, as well as for the shell, that we then used to create joint coordinate systems (JCSs) to describe the motions of one distal element relative to another, more proximal element (Brainerd et al., 2010; Menegaz et al., 2015). These JCS systems were established using Autodesk Maya and XROMM Maya Tools (D.B. Baier; xrommwiki.org), and the rotation order for calculating the Euler angles for all JCSs was ZYX in Maya. We measured pelvic girdle movements relative to the more proximal shell, and measured femoral movements relative to both the shell and the pelvis as more proximal elements. For each movement in each individual, we chose a neutral posture that we defined as a zero point.

For pelvic girdle movements, we defined our neutral posture as when the anterior tip of the pelvic midline pointed directly cranially. We created the pelvic ACS to be centered at the mediolateral midpoint of the pelvis, at the level of the center of the acetabulum. We aligned this ACS so that its x-axis was perpendicular to the plastron, its
y-axis passed through the acetabulum on both sides of the pelvis, and its z-axis pointed
directly craniocaudally. We then created a second ACS in the same location and
orientation that we parented to the shell, allowing the construction of a pelvic JCS to
measure relative movement of the distal pelvic ACS relative to the proximal shell ACS.
We arranged the pelvic JCS (Fig. 2a) so that roll, pitch and yaw of the pelvis were
measured as rotations about the x, y and z axes of the JCS, respectively, and
craniocaudal, mediolateral and dorsoventral translations were measured as translations
along the x, y and z axes of the JCS, respectively.
We defined our reference pose for femoral movements to be when the long axis of the
femur was perpendicular to the pelvis. We first created an ACS centered at the center of
the femoral head at the acetabulum. The x-axis of this ACS was aligned through the long-
axis of the femur, with the y-axis parallel to the ventral plane of the shell. At this
position, the femur was depressed by 8 - 10° relative to the body, and the trochanters
were aligned perpendicular to the ground. We then created a second ACS in the same
location and orientation and parented it to the shell, allowing the construction of a
femoral JCS to measure relative movement of the distal femoral ACS to the more
proximal shell ACS. To measure femoral movements relative to the pelvis, we also
created a third ACS in the same location and orientation, but parented it to the pelvis to
provide an alternative proximal ACS. We oriented our femoral JCS so that rotations
about the x-axis correspond to long axis rotation, rotations about the y-axis correspond
with elevation and depression, and rotations about the z-axis correspond to femoral
protraction and retraction (Fig. 2b).
To measure the translation of the dorsal tips of the ilia relative to the sacrum, we created an ACS on the sacrum in which the x-axis was aligned craniocaudally, the y-axis was aligned mediolaterally, and the z-axis was aligned dorsoventrally. We then attached a locator at the dorsal tip of the left ilium and output the motion of the locator relative to the body axes defined by the sacrum ACS. To visualize translations of the pubis and ischium over the interior surface of the plastron, we created motion trails for each bone and then measured the major and minor axes of these ovoid paths of motion.

**Precision analysis**

Precision of both marker tracking and animation were calculated following published protocols (Brainerd et al., 2010; Camp et al., 2014; Menegaz et al., 2015). Following data collection from live animals, we collected X-ray footage from a previously euthanized, frozen turtle that had been implanted with a similar set of markers, moving the specimen on the treadmill and through water at similar speeds to *in vivo* locomotion. Because the animal was frozen, any deviations from zero in distance between markers, and any apparent movement measured with JCSs could be considered a threshold for identifying noise in measurements for *in vivo* trials. Precision of marker tracking was obtained by calculating the standard deviation of mean inter-marker distance, while precision of animation was calculated as the average standard deviation of apparent angular movement for each bone (femur and pelvis).

**Statistical analyses**
Unless noted, all statistical analyses were performed in R (v 3.2.1, R Core Team, 2015). For both walking and swimming, pelvic girdle movements were compared between species using linear mixed effects models (lmer4 (Bates et al., 2015)) with species as a fixed effect and individual as a random effect. Within each species, comparisons of motions between walking and swimming were performed using linear mixed effects models with locomotor behavior as a fixed effect and individual as a random effect. Effect sizes based on mixed effects models were calculated following published methods (Xu, 2003). Femoral movements were further compared between species and environments using a canonical discriminant function analysis (CDA) in JMP (Version 11, SAS Institute Inc., Cary, NC 1989-2007). Variables included in the CDA were maximal and minimal long axis rotation (LAR), maximal elevation and depression, and maximal protraction and retraction. A Wilks-lambda test was performed to determine if the differences explained by the discriminant variables were significant. A multiple regression was conducted to evaluate the effect of stride frequency and femoral excursion on pelvic rotation of cryptodire turtles in each environment, using the degrees of femoral retraction per second and degrees of femoral excursion as predictor variables and pelvic yaw as a response variable.

RESULTS

Precision

Mean marker tracking precision was 0.1 mm for swimming and for walking. JCS translation precision was 0.4 mm for the femur and 0.5 mm for the pelvis while walking,
and 0.8 mm for the femur and 0.9 mm for the pelvis while swimming. Angular precision for JCS data for the femur was 0.22° during walking and 0.32° during swimming. Pelvic girdle rotation precision was 0.25° for walking and 0.27° for swimming.

Pelvic Motion

Pelvic motion differed substantially between the two species (Fig. 3, Table 1). The greatest difference was for yaw movements, in which the cryptodire pelvis rotated by 18.20° ± 0.45 and 8.75° ± 0.38 during walking and swimming (Appendix A - Movie 1), respectively. In contrast, the pleurodire pelvis did not yaw during walking or swimming (Table 1, Appendix A - Movie 2). The noise in mean pleurodire pelvic motions was approximately 0.1 deg (Supplemental Fig. S1), which is less than the 0.3 deg precision of our measurements, indicating that any motion that could be occurring was less than our ability to detect it. The pelvis also pitched and rolled in cryptodires, especially on land (pitch 2.57° ± 0.09, roll 3.49° ± 0.05); however, the pleurodire pelvic girdle did not rotate in either environment (Fig. 3, Fig. S1). Translations of the cryptodire pelvis were small, less than 1 mm in the craniocaudal and mediolateral directions, and less than 0.1 mm in the dorsoventral direction (Fig. 4). In general, all movements of the cryptodire pelvis were smaller during swimming than walking (Table 1, $p < 0.001$, $\Omega^2 > 0.45$).

Yawing of the pelvis inside the shell in cryptodires caused craniocaudal sliding of the dorsal tips of the ilia relative to their articulations with the sacrum. Craniocaudal ilium translation relative to the sacrum was 3.4 mm per step while walking and 1.9 mm per stroke while swimming ($p < 0.001$, $\Omega^2 = 0.833$). Pelvic yaw also caused the ventral
processes of the pubis and ischium to slide in elliptical patterns relative to the plastron (Appendix A - Movie 3). The pubis during walking circumscribed the largest ellipses, with major and minor axes of 7.4 and 2.4 mm. Smaller but still substantial sliding motions occurred between pubis and plastron during swimming and between ischium and plastron during walking and swimming (Table 2).

 Increased speeds in cryptodires led to increased pelvic girdle rotation while walking (multiple regression $p < 0.001$, $r^2 = 0.58$, $F = 35.49_{(2,51)}$) and swimming ($p = 0.001$, $r^2 = 0.39$, $F = 8.28_{(2,27)}$) (Fig. 5). During walking, pelvic yaw increased from 14° at the slowest speeds to 22° at the fastest speeds, and during swimming yaw increased from 6° at slow speeds to 14° at faster speeds (Fig. 5).

**Femoral kinematics**

In cryptodires, we measured the contribution of pelvic girdle rotations to femoral kinematics by comparing the movements of the femur relative to the shell with movements of the femur relative to the pelvis. This effectively let us ‘subtract’ pelvic girdle rotations from femoral kinematics. Pelvic movements contributed little to either elevation/depression of the femur or its axial rotation. However, pelvic yaw had a strong effect on femoral protraction/retraction excursions, contributing 9.86° ±1.49 on land and 7.64° ± 3.57 in water (paired t-test, $p < 0.001$, Cohens d = 0.90).

 Canonical discriminant function analysis identified two primary axes that cumulatively explained 95.16% of variation in femoral kinematics between species and across environments. Canonical 1 (C1, 68.86% of variance) was defined primarily by
differences between swimming and walking, whereas canonical 2 (C2, 26.25 % of variance) distinguished cryptodires from pleurodires (Fig. 6). Walking cycles were characterized by positive scores on C1, reflecting larger retraction angles and larger pronation values. Swimming cycles were characterized by negative scores on C1, reflecting low magnitudes of maximum elevation and supination (Table 3). On C2, pleurodires exhibit lower values of protraction (by 12.89° on land and 8.03° in water) and depression (by 4.84° on land and 2.56° in water) than cryptodires (Table 3). A Wilks’ lambda test indicated that the differences explained by the discriminant variables were significant (Wilks’ lambda = 0.16, p < 0.001).

Both taxa showed similar long axis rotation (LAR) excursions of the femur while walking (cryptodire = 34.32° ±1.30, pleurodire = 31.72° ± 1.13, p = 0.747, Ω² = 0.75). However, marked differences in LAR emerged between the two taxa during swimming (Table 3, Fig. 7). Pleurodires retained LAR during swimming that was only marginally lower (27.87° ± 1.93; p = 0.038, Ω² =0.25) than during walking; in contrast, cryptodires exhibit a substantial decrease in LAR by 12.5 degrees (to 18.4° ±1.08, p < 0.001, Ω² = 0.65) as they shift from walking to swimming (Fig. 7).

**DISCUSSION**

**Functional implications of pelvic movement for turtle locomotion**

Movements of the pelvic girdle play a crucial role in locomotion for a variety of tetrapods, improving locomotor performance by increasing femoral excursion and stride length (Jenkins, 1971; Pridmore, 1992; Reilly and Delancey, 1997; Reilly and Elias, 1998). Using XROMM, we were able to observe pelvic rotation during locomotion by a
species of cryptodire turtle in the directions of pitch, roll, and, most dramatically, yaw (Fig. 3). These results provide new insight into the locomotor function of turtles, as the pelvis had been considered immobile relative to the shell during earlier observations using standard X-ray visualization (Walker, 1971). Our data indicate that, despite being surrounded by a bony shell, cryptodire turtles move the pelvis in a fashion that is quite similar to many other vertebrates, with pelvic yaw in particular contributing to femoral excursion in both walking and swimming. Pelvic movements for cryptodires were greater during terrestrial walking than swimming, likely due to the greater reaction forces acting on the limb during the support of body weight on land (Blob et al., 2003; Butcher and Blob, 2008). However, in both environments, pelvic yaw for cryptodires increased with increasing speed. To the extent that greater speeds reflect efforts to achieve maximal performance, pelvic movements may make their greatest contribution to cryptodire locomotion under conditions in which animals face the highest performance demands.

With a joint-coordinate system located at the center of the pelvis (Fig. 2A), we found that the motion of the cryptodire pelvis was nearly pure rotation, with less than 1 mm of whole-pelvis translation. The main rotation was yaw, with small amounts of pitch and roll (Fig. 3). These rotations caused the dorsal tips of the ilia to slide relative to the sacrum, and the ventral tips of the ischia and pubis to slide in elliptical patterns relative to the plastron (Appendix A - Movie 3). Some of these sliding motions were quite substantial, typically more than 3 mm and up to 7 mm in the case of the pubis (Table 2). Many hip muscles originate on the cryptodire pubis (e.g. puboischiofemoralis internus) and ischium (e.g. the ventral head of the flexor tibialis complex) (Walker 1973), and
understanding how these muscles and their tendons interact with and influence the sliding of the pelvis relative to the shell at these joints is a promising area of future research.

In contrast to the prominent pelvic movements we observed in our cryptodire species, the derived fusion of the pelvis to both the carapace and plastron in pleurodire turtles appears to render their pelvis motionless relative to the shell during locomotion, as any movements in the pleurodire pelvis were smaller than our ability to detect them (<0.3 deg). This limitation of pelvic movement is correlated with smaller femoral protraction, in both walking and swimming, for pleurodires compared to cryptodires over the range of speeds that we observed. Whereas limits to limb excursion might typically be viewed as detrimental, they might not be disadvantageous for all types of locomotion. For example, during the limb-propelled rowing that most non-marine turtles use to swim (Rivera et al. 2011; Rivera et al., 2013), greater protraction of the hind limb could reduce the aquatic stability of turtles by exposing them to higher lateral forces during locomotion (Blob et al., 2003). Although more species need to be examined to test the generality of the patterns of femoral motion that we observed, if our measurements are representative of both lineages, pleurodires could be predicted to be more stable swimmers than cryptodires, potentially providing them with energetic and sensory advantages (Dougherty et al., 2010; Rivera et al., 2011). Evaluations of differences in swimming performance between pleurodires and cryptodires could help to inform understanding of the differences that have been recognized in the ecological distributions of these clades: whereas cryptodires have radiated onto land multiple times, pleurodires are all primarily aquatic (Bonin et al., 2006). Although factors that led to the derived fusion of the pelvis
to the shell in pleurodires are unresolved, data on swimming performance could provide a new context for considering the origin of their morphological novelty by indicating potential locomotor costs and benefits that might accompany any structural advantages of pelvic-shell fusion.

**Implications of pelvic and femoral movements for femoral loading mechanics**

Our data on femoral movements also indicated long axis rotation (LAR) of the femur that varied between taxa and habitats. Whereas pleurodires exhibited similar levels of LAR between swimming and walking, cryptodires showed a marked decrease in LAR during swimming (Fig. 6, Table 3). The functional role of limb bone LAR during locomotion is poorly understood. In birds, it has been shown to increase working space and maneuverability while walking (Kambic et al., 2014, 2015), whereas in sprawling taxa it has been suggested as a mechanism for increasing stride length (Rewcastle, 1983; Ashley-Ross, 1994; Reilly and Delancey, 1997). Regardless of its function, a consequence of femoral LAR identified during sprawling locomotion has been an elevation of torsional loads (Blob and Biewener, 1999, 2001; Butcher and Blob, 2008; Butcher et al., 2008; Sheffield et al., 2011; Blob et al., 2014). In this context, our data on pelvic and femoral movement in turtles are noteworthy in two regards. First, previous measurements of shear stresses (Butcher and Blob, 2008) and strains (Butcher et al., 2008) from *P. concinna* showed some of the highest torsional loads on the femur that have been found in walking vertebrates. This high torsion was attributed, in part, to the presumed immobility of the pelvis within the shell (Walker, 1971), which might have
required any twisting imposed on the body during locomotion to be accommodated by
the limb (Butcher and Blob, 2008). However, our finding that the pelvis is mobile in
cryptodires indicates an additional structure beyond the limbs that can help to
accommodate locomotor twisting to at least some degree, suggesting a greater role for
other mechanisms (e.g., immobile body axis, limb muscles: Butcher et al., 2008) that
might impose high torsion on cryptodire femora. In contrast, the lack of pelvic
movements in pleurodires might lead to elevated torsional loads on the femur for this
clade on land, perhaps limiting their terrestriality and contributing to their predominant
use of aquatic habitats.

A second insight into femoral loading mechanics in turtles provided by our
XROMM data relates to changes in loads between walking and swimming. In vivo strain
measurements from the femur of swimming cryptodires indicate a dramatic reduction in
shear strains during aquatic rowing compared to terrestrial walking, outpacing reductions
in bending loads (Young and Blob, 2015) and indicating that an additional factor, beyond
just the overall reduction in loads that accompanies aquatic body support from buoyancy,
is likely responsible. Our XROMM measurements provide evidence for such a factor, as
our cryptodires reduced femoral LAR during swimming by almost half in comparison to
walking (Fig. 6). The reduction of torsional loading in the proximal limb segments of
rowing cryptodires may have facilitated the evolution of flattened limb bones that are
typical of hyperspecialized flapping swimmers, such as sea turtles (Young and Blob,
2015). Beyond this possibility, our data indicate that the significant rotation of the foot
that occurs during the rowing strokes of turtles (Blob et al., 2008) must be largely
achieved through rotations of the distal limb segments – a prediction that could be tested through additional XROMM measurements.

**Concluding remarks**

Within the constraints of a bony shell, turtles exhibit considerable diversity in body plan and locomotor habits. Among cryptodire turtles in particular, species range from fully terrestrial taxa with high-domed shells, such as tortoises and box turtles, to highly aquatic species with flattened bodies, such as the trionychids (softshells). XROMM has, essentially, allowed the identification of a previously unknown mobile component in the locomotor apparatus of at least some members of the turtle lineage. The extent to which this component contributes to variation in the locomotor performance and ecology of these taxa remains to be explored, but such analyses carry strong potential to give insight into the functional diversity of this distinctive clade of animals and the diversity of pelvic girdle function across vertebrates more broadly.
ACKNOWLEDGEMENTS

We thank K. Diamond, J. Youngblood, J. Pruett, and A. Rubin for assistance with surgeries, B. Knörlein for his work designing XMA lab, R. Kambic for his expertise and assistance using Maya to make motion trails and render videos, H. and F. von Bülow for assistance with travel, E. Tavares for help with CT scans and housing arrangements for turtles during XROMM data collection, and two anonymous reviewers for their comments on the manuscript.

FUNDING

This work was supported by a Company of Biologists travel grant to C.J.M., Clemson Creative Inquiry funds (Grant #479) to R.W.B., and NSF 1262156 to E.L.B.
REFERENCES


Fig. 1. X-ray images of walking turtle in lateral (A) and ventral (B) views. Blue dots indicate markers on the pelvis, red dots are marker locations on the femur, and green dots are markers located on the shell (carapace and plastron).
Fig. 2. Joint coordinate systems (JCSs) used in this study from the pleurodire turtle, *Emydura subglobosa*. (A) Pelvic girdle joint coordinate system (dorsal view) for measuring motion of the pelvis relative to the shell. Axis orientations were set so that rotation about the $X$-axis (red) is roll, rotation about the $Y$-axis (green) is pitch, and rotation about the $Z$-axis (blue) is yaw. (B) Femoral joint coordinate system (posterior view). Axis orientations were set so that rotation about the $X$-axis (red) is long-axis rotation, rotation about the $Y$-axis (green) is elevation-depression, and rotation about the $Z$-axis (blue) is protraction-retraction. Femoral motion was measured relative to the pelvis and relative to the shell.
Fig. 3. Pelvic girdle rotations in cryptodire (*Pseudemys concinna*) and pleurodire (*Emydura subglobosa*) turtles during walking and swimming. Solid lines represent mean traces for each motion, shading represents standard errors for each motion, and colors indicate different axes of rotation (black - roll; blue - pitch; red - yaw). Traces were normalized to the same duration. Vertical dashed lines represent the transition from
stance to swing (walking) or from stroke to recovery (swimming). (A) Cryptodire pelvic rotations while walking (N = 3 individuals, 54 cycles). (B) Pleurodire pelvic rotations while walking (N = 2 individuals, 35 cycles). (C) Cryptodire pelvic rotations while swimming (N = 3 individuals, 30 cycles). (D) Pleurodire pelvic rotations while swimming (N = 2 individuals, 18 cycles).
Fig. 4: Cryptodire pelvic girdle translations during walking. Solid lines represent mean traces for each motion, shading represents standard errors for each motion, and colors indicate different axes of translation (black – craniocaudal; blue – mediolateral; red – dorsoventral). Vertical dashed line represents the transition from stance to swing. Traces from all trials were normalized to the same duration for calculation of mean kinematic profiles (N = 3 individuals, 54 cycles). Note that whole-pelvis translations are small, generally <1 mm.
Fig. 5. Multiple regression results illustrating the effect of increasing femoral excursion and stride frequency on pelvic rotation during walking (A), and swimming (B) in a cryptodire turtle, *Pseudemys concinna*. Points represent individual locomotor cycles on the regression surface (blue planes). Pelvic rotation increases significantly as excursion and frequency increase in both environments (Table 3).
Fig. 6. Canonical discriminant function analysis of femoral movements of cryptodire (*Pseudemys concinna*) and pleurodire (*Emydura subglobosa*) turtles. Colors of points indicate different categories of cycles: red, cryptodire walking; gold, cryptodire swimming; blue, pleurodire walking; purple, pleurodire swimming. Colored ovals represent 95% confidence limit for the mean of each of these groups. Black lines indicate the magnitude and direction of each variable (Youngerman et al., 2014). Locomotor behavior (Canonical 1) is loaded most strongly by increased retraction and pronation as well as negatively loaded by increased elevation and supination. Canonical 2 discriminates species, with decreased protraction and increased depression loading most heavily and separating pleurodires from cryptodires.
Fig. 7. Mean femoral long axis rotation (LAR) excursions of cryptodire (*Pseudemys concinna*) and pleurodire (*Emydura subglobosa*) turtles during walking and swimming. Error bars indicate ±1 SE. Pleurodires (circles) show substantially less femoral LAR during swimming compared to walking, whereas cryptodires (diamonds) show more similar femoral LAR excursions between behaviors.
Table 1. Mean (± SE) pelvic girdle rotations (deg) for cryptodire (*Pseudemys concinna*) and pleurodire (*Emydura subglobosa*) turtles during walking and swimming, with mixed effects model significance (p-value) and effect size ($\Omega^2$).

<table>
<thead>
<tr>
<th></th>
<th>Cryptodire</th>
<th>Cryptodire</th>
<th>Pleurodire</th>
<th>Pleurodire</th>
<th>Between species P- value</th>
<th>Between species $\Omega^2$</th>
<th>Cryptodire Between environment P- value</th>
<th>Cryptodire Between environment $\Omega^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>walk</td>
<td>swim</td>
<td>walk</td>
<td>swim</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3, 54)</td>
<td>(3, 30)</td>
<td>(2, 35)</td>
<td>(2, 18)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roll</td>
<td>3.49 ± 0.05</td>
<td>1.17 ± 0.06</td>
<td>0.29 ± 0.02</td>
<td>0.48 ± 0.05</td>
<td>&lt;0.001</td>
<td>0.97</td>
<td>&lt;0.001</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>0.97</td>
<td>0.05</td>
<td>0.02</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pitch</td>
<td>2.57 ± 0.09</td>
<td>1.18 ± 0.18</td>
<td>0.31 ± 0.02</td>
<td>0.39 ± 0.04</td>
<td>&lt;0.001</td>
<td>0.86</td>
<td>&lt;0.001</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yaw</td>
<td>18.2 ± 0.45</td>
<td>8.75 ± 0.38</td>
<td>0.61 ± 0.04</td>
<td>0.92 ± 0.09</td>
<td>&lt;0.001</td>
<td>0.98</td>
<td>&lt;0.001</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>0.87</td>
<td>0.04</td>
<td>0.04</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate samples sizes of (individuals, cycles) for each category.
Table 2: Major and minor axes of the elliptical sliding motions (in mm ± SE) of the cryptodire pubis and ischium relative to the plastron during walking and swimming.

<table>
<thead>
<tr>
<th></th>
<th>Pubis</th>
<th>Ischium</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Walk</td>
<td>Swim</td>
<td>Walk</td>
<td>Swim</td>
<td>Walk</td>
<td>Swim</td>
</tr>
<tr>
<td>Major</td>
<td>7.4</td>
<td>3.7</td>
<td>5.4</td>
<td>1.9</td>
<td>2.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Minor</td>
<td>± 1.5</td>
<td>± 0.4</td>
<td>± 0.2</td>
<td>± 0.5</td>
<td>± 0.3</td>
<td>± 0.3</td>
</tr>
</tbody>
</table>
Table 3. Mean femoral rotation (± SE) in each direction, relative to the neutral reference pose for cryptodire (*Pseudemys concinna*) and pleurodire (*Emydura subglobosa*) turtles during swimming and walking with discriminant analysis loadings.

<table>
<thead>
<tr>
<th></th>
<th>Cryptodire Walk (3, 54)</th>
<th>Cryptodire Swim (3, 30)</th>
<th>Pleurodire Walk (2, 35)</th>
<th>Pleurodire Swim (2, 18)</th>
<th>Canon 1 (68.86 % var)</th>
<th>Canon 2 (26.25% var)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supination (LAR)</td>
<td>-20.94 ± 0.88</td>
<td>-12.47 ± 1.90</td>
<td>-16.47 ± 0.78</td>
<td>-19.09 ± 3.63</td>
<td>-0.98</td>
<td>0.26</td>
</tr>
<tr>
<td>Pronation (LAR)</td>
<td>13.01 ± 1.43</td>
<td>5.92 ± 1.26</td>
<td>15.24 ± 0.79</td>
<td>8.78 ± 2.85</td>
<td>0.81</td>
<td>0.18</td>
</tr>
<tr>
<td>Maximum depression</td>
<td>-10.26 ± 0.50</td>
<td>-6.87 ± 0.80</td>
<td>-5.42 ± 0.34</td>
<td>-4.32 ± 0.82</td>
<td>0.39</td>
<td>0.60</td>
</tr>
<tr>
<td>Maximum elevation</td>
<td>4.89 ± 0.37</td>
<td>6.08 ± 1.43</td>
<td>5.76 ± 0.41</td>
<td>6.84 ± 1.05</td>
<td>-1.09</td>
<td>0.18</td>
</tr>
<tr>
<td>Maximum protraction</td>
<td>-83.77 ± 1.41</td>
<td>-75.95 ± 1.78</td>
<td>-70.88 ± 0.85</td>
<td>-67.92 ± 2.09</td>
<td>-0.08</td>
<td>0.58</td>
</tr>
<tr>
<td>Maximum retraction</td>
<td>15.76 ± 0.89</td>
<td>1.87 ± 2.63</td>
<td>26.96 ±2.03</td>
<td>6.33 ± 2.91</td>
<td>1.04</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Numbers in parentheses for each of the first four columns indicate samples sizes of (individuals, cycles) for each category.

Primary loadings for each canonical axis are in bold (Chan, 2003); canonical 1 distinguishes locomotor habitat, whereas Canonical 2 distinguishes the two lineages of turtles (Fig. 3).
CHAPTER THREE

HINDLIMB MUSCLE FUNCTION IN TURTLES: IS NOVEL SKELETAL DESIGN CORRELATED WITH NOVEL MUSCLE FUNCTION?

This is a pre-copy-editing, author-produced copy of an article accepted for publication in the Journal of Experimental Biology following peer-review. The definitive publisher-authenticated version:


Is available online at: http://jeb.biologists.org/content/220/14/2554.full-text.pdf?with-ds=yes

ABSTRACT

Variations in musculoskeletal lever systems have formed an important foundation for predictions about the diversity of muscle function and organismal performance. Changes in the structure of lever systems may be coupled with changes in muscle use and give rise to novel muscle functions. The two extant turtle lineages, cryptodires and pleurodires, exhibit differences in hindlimb structure. Cryptodires possess the ancestral musculoskeletal morphology, with most hip muscles originating on the pelvic girdle, which is not fused to the shell. In contrast, pleurodires exhibit a derived morphology, in which fusion of the pelvic girdle to the shell has resulted in shifts in the origin of most
hip muscles onto the interior of the shell. To test how variation in muscle arrangement might influence muscle function during different locomotor behaviors, we combined measurements of muscle leverage in five major hindlimb muscles with data on muscle use and hindlimb kinematics during swimming and walking in representative semiaquatic cryptodires and pleurodires. We found substantial differences in muscle leverage between the two species. Additionally, we found that there were extensive differences in muscle use in both species, especially while walking, with some pleurodire muscles exhibiting novel functions associated with their derived musculoskeletal lever system. However, the two species shared similar overall kinematic profiles within each environment. Our results suggest that changes in limb lever systems may relate to changes in limb muscle motor patterns and kinematics, but that other factors must also contribute to differences in muscle activity and limb kinematics between these taxa.

INTRODUCTION

Differences in structure across animal species are often used as a basis for predicting differences in their function (Hulsey et al., 2008; Anderson and Patek, 2015). One of the most common sources of structural variation that has been examined in this context is the leverage of muscle systems (Smith and Savage, 1956; Biewener, 1989; Westneat, 1994; Kargo and Rome, 2002). The leverage of muscles about joints can be compared through the measurement of moment arms, which are defined as the shortest distance between the line of action of a muscle-tendon complex and the center of rotation of a joint about
which the complex acts (Vogel, 2013). Muscles with larger moment arms generate a
greater moment about a joint for a given level of input force, reducing the absolute
amount of force that the muscle must generate to balance an external load (Hutchinson et
al., 2005). Yet, in addition to structural features, muscle function is also determined by a
wide range of dynamic components (Roberts et al., 1997; Ahn and Full, 2002), including
the timing of muscle activity (Biewener and Gillis 1999; Gillis and Blob, 2001). Thus,
correlations between changes in leverage and activity timing could profoundly affect the
functional roles of muscles throughout the evolution of a lineage (Lauder and Reilly,
1996). However, it is unclear if such correlations should be expected, given that
remarkably similar patterns of muscle activity have been documented for both locomotor
and feeding behaviors across taxa that exhibit highly divergent structures (e.g., Jenkins
and Goslow, 1983; Wainwright and Lauder, 1986; Westneat and Wainwright, 1989; Dial
et al., 1991).

Structural variations in the locomotor systems of turtles provide an opportunity to
specifically test for associations between changes in muscle leverage and changes in
muscle activation patterns. The two major lineages of turtles, cryptodires and pleurodires,
show differences in pelvic girdle structure that are correlated with differences in the
origins of many hindlimb muscles. Cryptodires possess the ancestral configuration, in
which the pelvis can move relative to the shell (Walker, 1973; Mayerl et al., 2016). In
contrast, pleurodires exhibit a derived condition, in which the pelvis has been fused to the
shell and become immobile (Walker, 1973; Mayerl et al., 2016). These skeletal changes
are associated with many of the muscles responsible for controlling the hindlimb shifting
from origins on the pelvis in cryptodires to origins on the interior surface of the shell in pleurodires (Fig. 1, Fig. S1; Walker, 1973). Because the moment arm of a muscle is strongly influenced by the location of its origin, these structural rearrangements of the pelvic girdle in pleurodires are likely to substantially change the leverage of the muscles that control hindlimb motion. Despite these differences in their anatomical structure, most freshwater turtles of both lineages show broadly similar patterns of hindlimb motion (e.g., using sprawling limb posture on land and rowing strokes in the water: Zug, 1971; Blob et al., 2008; Mayerl et al., 2016). Thus, changes in muscle leverage may not be reflected in their patterns of action. Alternatively, it is possible that the two lineages might activate their limb muscles differently but, with differences in muscle leverage, those activity patterns could lead to similar locomotor movements.

To investigate whether changes in limb muscle leverage are correlated with changes in muscle activity in turtles, we (1) compared the leverage of five major hindlimb muscles between representative semiaquatic cryptodire (*Trachemys scripta*) and pleurodire (*Emydura subglobosa*) species, and (2) used electromyography (EMG) to measure the activity patterns of these muscles while (3) recording hindlimb kinematics during swimming and walking. We found that changes in muscle leverage may contribute to novel muscle use in pleurodires, but that some pleurodire limb muscles show novel patterns of activity even without changes to their anatomical leverage. Additionally, our results indicate that hindlimb kinematics differ strongly between locomotor environments (i.e., water versus land) in both turtle lineages, but that cryptodires and pleurodires may utilize similar kinematics in each environment through different patterns of muscle use.
MATERIALS AND METHODS

Experimental animals

Six adult Jardine River Turtles (16.5 – 22.3 cm), *Emydura subglobosa*, Krefft (1880) were purchased from a commercial supplier (Turtles and Tortoises Inc., Brooksville, FL). Three adult red eared sliders (18.5 – 19.5 cm), *Trachemys scripta*, Shoepff (1792) were collected with hoop traps from a spillway of Lake Hartwell, Pickens county, SC, USA (South Carolina Scientific Collection permit #28-2016). We also supplemented our cryptodire data with results from two additional individuals that were published in a previous study (Blob et al., 2008). Turtles were housed in pairs in 600-liter stock tanks equipped with pond filters, a submerged 200-watt heater (maintaining water at 25 deg C), and dry basking platforms. Tanks were located in a temperature controlled greenhouse facility, exposing turtles to ambient light patterns throughout the course of experiments (February 2015 to August 2016). Turtles were fed a diet of commercial pellets (ReptoMin®, Tetra®, Blacksburg, VA, USA), supplemented with earthworms. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Clemson University (protocols 2013 – 051, 2014 – 079).

Collection and analysis of muscle properties

Although our measurements of muscle structure were the last chronological stage in our study, we will describe these methods first because their results provide the primary context for interpreting our data on muscle activity patterns and limb movements. At the
conclusion of data collection for muscle use and kinematic patterns, experimental animals were euthanized and the muscles of the right (non-instrumented) hindlimb were dissected to determine their moment arms and physiological cross sectional areas (PCSA). Focus muscles included puboischiophemoralis internus (PIFI, Fig. 1 A,B), iliofemoralis (ILF, Fig. 1 C,D), femorotibialis (FT, Fig. S1), the dorsal and ventral heads of the flexor tibialis internus (FTI-D and FTI-V, Fig. 1 E,F), and caud-iiliofemoralis (CIF, Fig. 1 G,H). FTI is characterized by possessing a dorsal head, which originates on the sacral vertebrae in both lineages, and a smaller ventral head, which originates on the puboischiadic ligament and the border of the ischium in cryptodires, but originates on the plastron in pleurodires (Walker, 1973). Because of these differences, we collected moment arm data on both heads of this muscle separately, although we were not able to collect motor pattern data from the ventral head. Based on the anatomical locations of these five muscles and previous studies of cryptodire turtles, PIFI and ILF are considered the primary hip protractors, FT is regarded as a knee extensor, FTI is considered a hip retractor and knee flexor, and CIF is considered a hip retractor (Walker, 1973, Blob et al., 2008; Aiello et al., 2013).

Muscle measurements were collected from all six E. subglobosa used to collect muscle activity and kinematic data, as well as from six previously euthanized T. scripta that had been frozen after earlier experiments (Rivera and Blob, 2010). To identify the joint center of rotation, we palpated and manipulated the leg and placed a metal pin at the center of rotation of the hip. We then observed the location of the pin to ensure that no translation occurred as the limb was manipulated. To quantify the moment arm of a
muscle about a joint, we used digital calipers to measure the perpendicular distance from
the joint to the midline of each focus muscle spanning the joint in the directions of hip
abduction/adduction, hip protraction/retraction, and knee flexion/extension, as applicable.
Moment arms for the hip were measured with the limb parallel with the plane of the
plastron at 45, 90, and 135 degrees of protraction/retraction, relative to the cranial axis of
the body. For flexion and extension of the knee, we measured moment arms at maximal
flexion and extension, as well as when the crus was positioned perpendicular to the thigh.
These measurements represented the moment arms of each muscle throughout a complete
limb cycle. For each muscle, we then used the average of the three measurements for
each variable in statistical analyses. To compare hindlimb muscle leverage across
differently sized individuals and provide a better reflection of muscle mechanical
advantage at joints, we converted our moment arm measurements into size normalized
moment arms by dividing the moment arm by femur length for moments occurring at the
hip, and by tibia length for moments occurring at the knee.

After joint moment arms were measured, muscles were dissected out from each
turtle. Muscle fascicle lengths were determined by measuring the length of fascicles at
the center of the muscle when the muscle was laid flat on the dissecting tray, and muscles
were weighed using a digital scale. PCSA was calculated following standard protocols
(Biewener and Full, 1992). To account for minor differences in size across individual
turtles, PCSA was normalized for comparisons by dividing raw PCSA by the square of
the length of the femur.
Collection and analysis of electromyographic data

Electromyographic (EMG) data were collected from five focal hindlimb muscles (PIFI, ILF, FT, FTI-D, and CIF). These muscles are thought to be the primary muscles used to power hindlimb movements in turtles, are the largest muscles in the hindlimb, and cover all major planes of hindlimb motion during locomotion (Walker, 1973; Blob et al., 2008). Additionally, limited data for these muscles have been collected from cryptodire turtles in previous experiments (Blob et al., 2008; Aiello et al., 2013), providing comparisons to our new recordings. Data were collected during both swimming and terrestrial walking behaviors in a custom-built, recirculating flow tank. For aquatic trials, turtles were induced to swim by adjusting flow speed to elicit constant swimming behavior against flow (Pace et al., 2001). In walking trials, turtles were filmed walking over a clear Plexiglas platform into a water-filled refuge. As in previous work, a transparent surface was required to collect kinematic data, but the dried Plexiglas surface did not inhibit terrestrial locomotion (Rivera et al., 2010). For each turtle, 25-30 limb cycles were collected for each locomotor behavior.

To ensure accurate placement of electrodes, we performed dissections on previously euthanized individuals in order to determine external landmarks for EMG implantation. Prior to implantation, we induced analgesia and anesthesia with intramuscular injections of 1 mg kg$^{-1}$ butorphenol, 30 mg kg$^{-1}$ ketamine, and 1mg kg$^{-1}$ xylazine into the right forelimb, following published procedures (Rivera et al., 2010; 2011; 2013). While anesthetized, anatomical landmarks were marked for kinematic measurements (see below), and bipolar fine-wire electrodes (0.05 mm diameter, insulated
stainless steel; 0.5mm barbs; California Fine Wire Co., Grover Beach, CA, USA) were
implanted percutaneously into target muscles in the left hindlimb using hypodermic
needles. For each experiment, up to 15 implants were performed, with target muscles
receiving between 2-4 electrodes to ensure successful recordings even if some electrodes
failed. Electrode wires were glued together into two cables, with wires inserted into
posterior muscles (CIF and FTI-D) being grouped into one cable, and wires inserted into
anterior muscles (PIFI, ILF, and FT) grouped into the other
cable. These cables were
given several centimeters slack, and were then secured to the carapace with waterproof
tape. Turtles were then allowed to recover from anesthesia overnight.

During locomotor trials, EMG signals were relayed from electrodes in turtles to a
Grass 15LT amplifier system (West Warwick, RI, USA) for amplification (10,000 times)
and filtering (60 Hz notch filter, 30 Hz – 6kHz bandpass). Analog EMG signals were
converted to digital data and collected at 5000 Hz using custom LabVIEW (v.6.1;
National Instruments Corp., Austin, TX, USA) routines. Following data collection, turtles
were euthanized by an intraperitoneal injection of sodium pentobarbital (200 mg kg-1)
and experimental limbs were dissected to verify electrode placement.

EMG data were synchronized with limb kinematics by triggering a signal
generator that simultaneously produced a square wave in the EMG data and a light pulse
visible in the video. EMGs were then analyzed using custom LabVIEW software routines
to identify bursts of muscle activity. The variables calculated included the percentage of
the cycle at which muscle activity began (onset) and ended (offset). The number of trials
from which EMG data were collected varied across individuals and muscles due to differences in electrode implant success.

*Collection and analysis of kinematic data*

Coincident with EMG data collection, kinematic data were collected at 100 Hz using two digitally synchronized high-speed video cameras (Phantom V5.1, Vision Research, Inc.; Wayne, NJ, USA). Lateral and ventral views were collected simultaneously, with the ventral view obtained by directing the camera at a mirror angled at 45 degrees with respect to the transparent bottom of the flow tank arena.

To facilitate measurement of kinematics, anatomical landmarks (13 ventral; 9 lateral) were marked with non-toxic white nail polish, with a smaller, black point painted in the center of the white dot to create trackable, high-contrast points (Fig. 2). The landmarks used in both views included: tip of the nose, hip, knee, ankle, digits 1, 3, and 5, and anterior and posterior points on the bridge of the shell. We also marked points on the right, left, anterior, and posterior margin of the plastron that were visible in ventral view. Landmarks were tracked using DLTDataviewer5 (Hedrick, 2008), and the resulting three-dimensional coordinate data were processed using custom MatLab routines to determine kinematic excursions during swimming and walking (Rivera et al., 2010). Kinematic data included femoral protraction and retraction angles, femoral elevation and depression angles, extension and flexion angles of the knee and ankle, and the rotation (feathering) angle of the pes. These variables were then processed through a quintic spline to smooth and interpolate the data to 101 values, representing 0 – 100% of
the limb cycle (0 being a fully protracted limb). These procedures facilitated comparisons of kinematic profiles for locomotor cycles of different absolute durations.

Kinematic angles were defined as follows. A femoral protraction/retraction angle of 0 deg indicates a femur perpendicular to the midline of the body, where positive values indicate a more protracted femur, and negative values indicate greater retraction. A femoral elevation/depression angle of 0 deg indicates the femur is in the horizontal plane of the turtle, where positive angles indicate elevation and negative angles indicate depression. A knee extension/flexion angle of 0 deg would indicate a fully flexed knee, whereas a 180 deg angle would indicate a fully extended knee. An ankle angle of 0 deg would indicate the pes was dorsiflexed to fold on top of the distal crus, whereas an angle of 90 deg would indicate that the pes was held perpendicular to the crus, and an angle of 180 deg would indicate that the pes was parallel to and extending from the distal crus. Pes rotation (feathering) angle was calculated as the angle between a vector pointing along the anteroposterior midline and a vector emerging from the plantar surface of the pes, which was defined by the points marking the ankle and the tips of digits 1 and 5. This angle was transformed by subtracting 90 deg from each value (Pace et al., 2001). A high drag orientation of the pes, with the paddle directed perpendicular to the direction of travel, is indicated by an angle of 90 deg, whereas a low drag orientation of the pes is indicated by an angle of 0 deg.

*Statistical analysis*
Unless otherwise noted, all statistical analyses were performed in R (v 3.2.1, www.r-project.org).

**Muscle anatomy**

Anatomical properties for the muscles we measured from both turtle lineages, including size normalized moment arm for each direction of motion for each muscle, as well as size-normalized PCSA, were compared using Cohen’s d tests (effsize; Torchiano, 2016), which evaluate the difference between two means divided by the standard deviation of the data (Cohen, 1992). This provides an effect size estimate of the treatments (in this case, the difference between the two species). We considered any variables with a Cohen’s d of greater than 1 to be different between the two lineages, a conservative estimate for considering large effect sizes (Cohen, 1992).

**EMGs**

We performed a canonical discriminant analysis (CDA) using onset and offset timings for each muscle in each environment to determine the primary variables explaining the variation between species and environments (MASS; Venables and Ripley, 2002). A Wilks-lambda test was performed to determine if the differences explained by the discriminant variables were significant. Due to electrode and equipment failures, we do not have data for every muscle from each individual. Data included in the analysis were mean muscle onset and offset timings of each muscle in each species (Table S1).
Kinematics

Differences in kinematics between environments and species were compared using a canonical discriminant analysis (CDA) (MASS; Venables and Ripley, 2002). Variables included in the CDA were maximal protraction and retraction angles, maximum abduction and adduction angles, maximum knee flexion and extension angles, maximum ankle flexion and extension angles, and maximum and minimum pedal feathering angles. A Wilks-lambda test was performed to determine if the differences explained by the discriminant variables were significant.

We further used linear mixed effects models for each set of kinematics (lmer4; Bates et al., 2015) to test for differences between species within an environment. We used species as a fixed effect, and individual and trial (intercept varying within trial) as random effects (X ~ species + (1|individual/trial). P-values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question. Effect sizes based on mixed effects models were calculated following published methods (Cohen, 1992; Xu, 2003).

RESULTS

Muscle leverage and size

We found that muscles that showed no change in origin between the two lineages (FT and FTI-D; Fig. 1, Fig. S1) showed no difference in size normalized moment arm for any moment (Table 1). In contrast, the other four muscles (PIFI, ILF, FTI-V, and CIF; Fig. 1) all showed greater size normalized moment arms in abduction/adduction in pleurodires.
than they did in cryptodires (Table 1; Fig. 3). When normalized by femur length, PCSA was similar between lineages for all muscles. We found very few changes in sized normalized moment arms for protraction/retraction or flexion/extension, although size normalized moment arms for protraction in PIFI and retraction in CIF were larger in pleurodires than cryptodires (Table 1).

Muscle activity patterns
In each lineage, we found substantial differences in muscle activity between swimming and walking (Fig. 4, Table S2). Comparing patterns within each environment between the lineages, the specific timings of onset and offset differ in some muscles during swimming, but they are active during the same general periods of activity in both cryptodires and pleurodires (Fig. 4A). In contrast, during walking, larger differences are exhibited between the lineages, with substantial changes in activity timing for most muscles, and generally increased duration of activity in pleurodires (Fig. 4B). Pleurodires exhibit bursts of activity during both stance and swing for FT that are similar to the two burst pattern for this muscle found in cryptodires (Blob et al., 2008). However, pleurodires also exhibited an unexpected second burst of activity by PIFI during the stance phase of walking that has not been observed in walking cryptodires (Fig. 4B).

Canonical discriminant function analysis identified two axes of variation that together explained 98.02% of variation in EMG patterns between species and across environments (Fig. 5). Canonical 1 (C1, 75.01% of variation) separated pleurodire walking motor patterns from all other motor patterns. Walking pleurodires were
particularly distinguished by later offset timing of CIF, FTI, and ILF, as well as later onset timing of PIFI (Table S2). Canonical 2 (C2, 23.01% of variation) separated pleurodires swimming from cryptodire walking and swimming. Pleurodires swimming was characterized primarily by later offset of FT (Table 2). A Wilks’ lambda test indicated that the differences explained by the discriminant variables were significant (Wilks’ lambda = 0.007, p < 0.001).

Kinematics

We found no differences in velocity between the two species during swimming (T. scripta: 1.59 ± 0.02 BL/s, E. subglobosa: 1.57 ± 0.02 BL/s, p = 0.547, Cohen’s d = 0.141, $\Omega^2 = 0.396$), or walking (T. scripta: 0.53 ± 0.04 BL/s, E. subglobosa: 0.47 ± 0.02 BL/s, p = 0.987, Cohen’s d = 0.207, $\Omega^2 = 0.253$). The two species spent similar amounts of time during thrust and recovery while swimming, with both lineages shifting from retraction to protraction at roughly 45% of the limb cycle. However, while walking, cryptodires began femoral protraction at approximately 61% of the cycle, whereas pleurodires did not begin femoral protraction until 73% of the cycle (Fig. 6).

In addition to differences in the timing of limb movements, pleurodires and cryptodires also differed in the extent of limb movement. In both swimming and walking, cryptodires protracted their femora to a greater degree than pleurodires, but retracted them less (Fig. 6, Table S3). Cryptodires also depressed their femur less than pleurodires, and extended their knee less (Fig. 6, Table S1). There was little difference in ankle
extension or flexion between the two lineages in either environment (Fig. 6, Table S1). However, during swimming, pleurodires orient the paddle of their pes in a higher drag position than cryptodires during thrust (Fig. 6, Table S1).

Canonical discriminant function analysis identified two primary axes that together explained 97.41% of variation in hind limb kinematics between species and across environments. Canonical 1 (C1, 87.98% of variance) was defined primarily by differences between walking and swimming, whereas canonical 2 (C2, 9.43% of variance) distinguished cryptodires from pleurodires (Fig. 7). Walking cycles were characterized by positive scores on C1, reflecting larger protraction angles, decreased knee flexion, and decreased pes rotation (during swing). Swimming cycles were characterized by negative scores on C1, reflecting increased pes rotation angle (foot oriented in ‘high drag’ position, occurring during thrust). Thus, during swimming, there is greater movement in the distal elements of the hindlimb as the animal produces drag-based propulsion, whereas during walking, the limb is protracted further to extend the stride. On C2, pleurodires are distinguished by decreased knee flexion and increased ankle extension. A Wilks’ lambda test indicated that the differences explained by the discriminant variables were significant (Wilks’ lambda = 0.16, p < 0.001).

DISCUSSION

The fusion of the pelvis to the shell in pleurodire turtles has resulted in derived locations of origin for muscles that power their hindlimb movements (Walker, 1973). These shifts in muscle origin have resulted in pleurodires exhibiting greater size normalized moment
arms for femoral adduction and abduction in these muscles when compared to cryptodire
turtles (Table 1). We found that some muscles that exhibit drastic changes in muscle
leverage between these lineages show changes in motor pattern, especially while
walking. Additionally, some muscles that show little anatomical difference between the
two lineages can also show differences in hindlimb muscle use during locomotion. Thus,
pelvic girdle fusion and the subsequent muscular reorganization of the hindlimb muscles
may account for some differences in limb function between cryptodire and pleurodire
turtles, but other dynamic components of muscle function likely also contribute to
differences in limb muscle use between these lineages (Roberts et al., 1997; Ahn and
Full, 2002).

In pleurodires, all muscles that have experienced a shift in origin also demonstrate
greater size normalized moment arms for ab/adduction. This change in leverage does not
appear to influence muscle use during swimming for the semiaquatic turtle species we
studied, as both lineages exhibited similar patterns of muscle activity in water (Fig. 4A).
However, when these turtles moved on land, we observed substantial differences in
muscle use between the taxa (Fig. 4B). In some cases, the shifts in leverage due to girdle
fusion may contribute to novel functional capacities for specific muscles. For example,
PIFI is typically regarded as a femoral protractor in cryptodires (Walker, 1973; Blob et
al., 2008). However, PIFI has approximately triple the sized normalized moment arm for
adduction in pleurodires than cryptodires, and shows a novel burst of activity during the
femoral retraction (stance) phase of walking in pleurodires, during which femoral
adduction is greater than in cryptodires (Fig. 6D). This burst of muscle activity in PIFI
corresponds with a delayed onset of the hip retractor CIF during pleurodire walking (Fig. 4B). In CIF, the pleurodire size normalized moment arm for abduction was also over triple what was observed in cryptodires (Table 1). Thus, we see substantial changes in leverage in these two muscles, which also show drastic differences in muscle activity between the two lineages while walking. The novel burst of activity in PIFI could potentially be functioning to counteract the increased duration of CIF activity, supporting the body during stance as the limb is retracted.

Although some differences in hindlimb motor patterns and kinematics between pleurodires and cryptodires, like those for PIFI, might relate to the differences in leverage imposed by pelvic-shell fusion, other differences in hindlimb muscle activity and kinematics are likely independent of the structural differences between the two lineages. For example, knee kinematics (Fig. 6E, F), and FT muscle activity (Figs. 4, 5), differ substantially between pleurodires and cryptodires. These differences are apparent even though FT does not cross the hip, and its disposition is not affected by the presence or absence of pelvic-shell fusion. These results indicate that the substantial anatomical changes in the lever systems of turtle hindlimbs are not the only factor which may result in novel patterns of muscle use. Moreover, in the absence of structural changes, kinematic differences between groups can be expected to be driven by differences in motor pattern (Smith, 1994).

Overall, we found that species (pleurodire versus cryptodire) had the greatest influence in defining patterns of hindlimb muscle use in semiaquatic turtles (Fig. 5), whereas environment (water versus land) had the greatest influence in defining their
patterns of kinematics (Fig. 7). With regard to muscle activity patterns, pleurodire walking differed from all other groups along the primary axis of variation in multivariate analyses, and pleurodire swimming separated from cryptodire walking and swimming along the second axis of variation (Fig. 5). In contrast, differences between walking and swimming explained the majority of kinematic variation in our sample, with some of the main features contributing to this distinction relating to variables that likely have little connection to differences in pelvic muscle moment arms between the groups (e.g., pedal feathering angle) (Fig. 7). Our multivariate results reinforce the dramatic kinematic differences required to produce effective locomotion between water and land in both species, regardless of differences in muscle use between species (Gillis and Blob, 2001; Nauwelaerts and Aerts, 2003; Rivera and Blob, 2010). Thus, changes in limb structure may relate to changes in limb muscle motor pattern and locomotor behavior in some cases, but these levels of variation also seem to show considerable independence (Smith, 1994; Lauder and Reilly, 1996).

Although not all differences in activity patterns between pleurodire and cryptodire hindlimb muscles were correlated with differences in muscle leverage, it is striking that, for muscles that did differ in leverage between our focal taxa, pleurodires always showed greater normalized muscle moment arms than cryptodires (Table 1). This result makes a noteworthy parallel with Walker’s (1973) comparisons of the shoulder muscles of a variety of turtle taxa, in which he found that the shoulder muscles of aquatic turtles exhibit greater mechanical advantage than those of their terrestrial relatives. Elevated
moment arms may help habitually aquatic, limb-propelled swimmers to produce force-generating movements within the compliant environment of water.

In this context, it is notable that pleurodire turtles, including *E. subglobosa*, have remained a primarily aquatic lineage throughout their evolutionary history, whereas cryptodires have radiated onto land multiple independent times (Joyce and Gauthier, 2003; Bonin et al., 2006). In addition to being affected by differences in muscle attachment, muscle function also can be influenced by the external environment (Gillis and Blob, 2001; Nishikawa et al., 2007; Foster and Higham, 2017; Janshen et al., 2017), and animals often exhibit differences in both the timing (Gillis and Biewener, 2000; Blob et al., 2008), and intensity (Biewener and Gillis, 1999; Gillis and Biewener, 2000) of muscle use during locomotion in water and on land. In addition to structural variations, such as differences in muscle moment arms, such dynamic modulations of muscle activity are likely an important component of motor control that allows animals to use the same structures to move through different environments (Gillis, 1998; Earhart and Stein, 2000; Rivera et al., 2010; Ashley-Ross et al., 2014; Perlman and Ashley-Ross, 2016).

Finally, our data may also provide insight into aspects of the water-to-land transition in vertebrates. The capacity for performance in multiple environments may be facilitated because changes in limb function are not necessarily related to changes in limb structure (Gillis and Blob, 2001). As a result, the initial invasions of land may have proceeded with structures used in aquatic environments, with muscle use being modulated before structural changes occurred (Cole et al., 2011; Boisvert et al., 2013; Kawano and Blob, 2013; Horner and Jayne, 2014; McInroe et al., 2016).
ACKNOWLEDGEMENTS

We thank A. Arellanez, K. Diamond, J. Youngblood, A. Rubin, C. Petty, and A. Sansone for assistance with surgeries and data collection, M. Sears and S. Kawano for their assistance with statistics, and two anonymous reviewers for their comments on the manuscript.

FUNDING

This work was supported by a Sigma Xi grant to C.J.M., and Clemson Creative Inquiry funds (Grant #479) to R.W.B.

DATA AVAILABILITY

Turtles used in this study are held in the collections of the Campbell Museum of Natural history at Clemson University. Data used in analyses are available at the Dryad digital repository (https://doi.org/10.5061/dryad.8j413).
REFERENCES


McInroe, B., Astley, H. C., Gong, C., Kawano, S. M., Schiebel, P. E., Rieser, J. M.,


FIGURES AND TABLES
Fig. 1 – Isolated hindlimb muscles of turtles that have experienced a change in their location of origin associated with pelvic girdle fusion in the pleurodire lineage.

Muscles have shifted from the ancestral origin on the pelvis in cryptodires (right, *T. scripta*), to the shell in pleurodires (left, *E. subglobosa*). (A,B) Puboischiofemoralis internus (PIFI, yellow); (C,D) Iliofemoralis (ILF, red); (E,F) Flexor tibialis interus (FTI, green); (G,H) Caudo-iliofemoralis (CIF, purple). Darker shading represents locations of muscle origin and insertion. Femorotibialis (FT) is not illustrated because it does not cross the hip and is thus not directly affected by pelvic girdle fusion.
Fig. 2. Representative still images from lateral (A) and ventral (B) views showing landmarks digitized for kinematic analysis. Points 1-9 are the same in both views; points 10-13 are only visible in ventral view. Landmarks: 1, tip of nose; 2, hip; 3, knee; 4, ankle; 5, digit 1; 6, digit 3; 7, digit 5; 8, anterior point on bridge; 9, posterior point on bridge; 10, point on left side of plastron; 11, point on right side of plastron; 12, posterior point on plastron; 13, anterior point on plastron
Fig. 3. Size normalized moment arms in ab/adduction for the five focal hindlimb muscles for each species (grey circle = cryptodire, *Trachemys scripta* (N = 6 individuals); black diamond = pleurodire, *Emydura subglobosa* (N = 6 individuals)). The femorotibialis (FT) is not plotted because it does not exert moments about the hip. PIFI add, Puboischiofemoralis internus adduction; ILF Ab, Iliofemoralis abduction; FTID Ab, Flexor tibialis internus (dorsal head) abduction; FTIV Add, Flexor tibialis internus (ventral head) adduction; CIF Ab, Caudoiliofemoralis abduction. For all muscles that have experienced a shift in origination (all but FTID), the pleurodire exhibits greater ab/adduction than the cryptodire.
Fig. 4. Hindlimb muscle use while swimming (A) and walking (B) in cryptodire (*T. scripta*) (black) and pleurodire (*E. subglobosa*) (blue) turtles. Bars represent the mean and standard error for the period of activity for each muscle. Vertical lines indicate the switch from retraction to protraction for each lineage for each behavior. See Table S1 for sample sizes for each muscle in each habitat for each species.
Fig. 5. Canonical discriminant function analysis of hindlimb muscle activity patterns in cryptodire (*T. scripta*) and pleurodire (*E. subglobosa*) turtles. Muscle use is defined primarily by species, with walking pleurodires showing the most distinct patterns along the first axis (Canonical 1), and swimming pleurodires showing the most distinct patterns along the second axis (Canonical 2). Colors of points indicate different cycle categories: red, cryptodire swimming (N = 33); yellow, pleurodire swimming (N = 28); light blue, cryptodire walking (N = 32); purple, pleurodire walking (N = 23). Colored ovals represent the 95% confidence limit for the mean of each of these three groups. Black lines indicate the magnitude and direction of the loading of each variable (Youngerman et al., 2014). Pleurodire walking (canonical 1) differentiated from the other
three groups, and is represented primarily by delayed offset of CIF, FTI and Il Fem, as well as delayed onset of PIFI. Canonical 2 discriminates pleurodires swimming from cryptodire walking and swimming, and is loaded most strongly by later onset of CIF (caudoiliofemoralis), and later offset of FT (femorotibialis). See Table 2 for loadings.
Fig. 6. Mean hindlimb kinematics of cryptodire (*T. scripta*, black) and pleurodire (*E. subglobosa*, blue) turtles while swimming (left, cryptodire: N = 5 individuals, 84 cycles; pleurodire: N = 6 individuals, 149 cycles) and walking (right, cryptodire: N = 5 individuals, 88 cycles; pleurodire: N = 6 individuals, 116 cycles). A,B, Hip
protraction/retraction; C,D, Hip elevation/depression; E,F, Knee flexion/extension; G,H, Ankle flexion/extension; I,J, Paddle orientation. Opaque lines indicate the mean value throughout the limb cycle, with shaded lines indicating standard error of the mean throughout the limb cycle.
Fig. 7. Canonical discriminant function analysis of hindlimb kinematics in cryptodire (*T. scripta*) and pleurodire (*E. subglobosa*) turtles. Kinematics are defined primarily by the environment the animal is experiencing. Colors of points indicate different cycle categories: red, cryptodire swimming (N = 84); yellow, pleurodire swimming (N = 149); blue, cryptodire walking (N = 88); purple, pleurodire walking (N = 116). Colored ovals represent the 95% confidence limit for the mean of each of these three groups. Black lines indicate the magnitude and direction of the loading of each variable (Youngerman et al., 2014). Canonical 1 is defined by differences in swimming and walking, with increased feathering defining swimming, and greater protraction angles.
defining walking. Canonical 2 discriminates species, with pleurodires exhibiting decreased knee flexion and increased ankle extension.
Table 1. Differences between cryptodire and pleurodire turtles in normalized muscle physiological cross sectional areas (PCSA) and size-normalized moment arm (MA) for directions of hindlimb motion.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Variable</th>
<th>Cryptodire</th>
<th>Pleurodire</th>
<th>Cohen’s d</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIFI</td>
<td>PCSA</td>
<td>$7.55 \times 10^8 \pm 3.63 \times 10^{-9}$</td>
<td>$7.59 \times 10^8 \pm 1.19 \times 10^{-8}$</td>
<td>$=$</td>
</tr>
<tr>
<td></td>
<td>MA-Pro/ret</td>
<td>0.145 $\pm$ 0.012</td>
<td>0.188 $\pm$ 0.015</td>
<td>$+$</td>
</tr>
<tr>
<td></td>
<td>MA-Ab/add</td>
<td>0.057 $\pm$ 0.005</td>
<td>0.150 $\pm$ 0.030</td>
<td>$+$</td>
</tr>
<tr>
<td>ILF</td>
<td>PCSA</td>
<td>$4.21 \times 10^8 \pm 5.72 \times 10^{-9}$</td>
<td>$5.33 \times 10^8 \pm 1.19 \times 10^{-8}$</td>
<td>$=$</td>
</tr>
<tr>
<td></td>
<td>MA-Pro/ret</td>
<td>0.131 $\pm$ 0.011</td>
<td>0.173 $\pm$ 0.029</td>
<td>$=$</td>
</tr>
<tr>
<td></td>
<td>MA-Ab/add</td>
<td>0.127 $\pm$ 0.022</td>
<td>0.273 $\pm$ 0.028</td>
<td>$+$</td>
</tr>
<tr>
<td>FT</td>
<td>PCSA</td>
<td>$3.66 \times 10^8 \pm 3.11 \times 10^{-9}$</td>
<td>$4.98 \times 10^8 \pm 9.62 \times 10^{-9}$</td>
<td>$=$</td>
</tr>
<tr>
<td></td>
<td>MA-Flex/Extend</td>
<td>0.121 $\pm$ 0.021</td>
<td>0.106 $\pm$ 0.007</td>
<td>$=$</td>
</tr>
<tr>
<td>FTI-D</td>
<td>PCSA</td>
<td>$3.92 \times 10^8 \pm 4.29 \times 10^{-9}$</td>
<td>$5.43 \times 10^8 \pm 7.34 \times 10^{-9}$</td>
<td>$=$</td>
</tr>
<tr>
<td></td>
<td>MA-Pro/ret</td>
<td>0.278 $\pm$ 0.025</td>
<td>0.271 $\pm$ 0.017</td>
<td>$=$</td>
</tr>
<tr>
<td></td>
<td>MA-Ab/add</td>
<td>0.100 $\pm$ 0.016</td>
<td>0.119 $\pm$ 0.011</td>
<td>$=$</td>
</tr>
<tr>
<td></td>
<td>MA-Flex/Extend</td>
<td>0.169 $\pm$ 0.025</td>
<td>0.156 $\pm$ 0.013</td>
<td>$=$</td>
</tr>
<tr>
<td>FTI-V</td>
<td>PCSA</td>
<td>$1.75 \times 10^5 \pm 3.48 \times 10^{-6}$</td>
<td>$5.23 \times 10^5 \pm 4.13 \times 10^{-6}$</td>
<td>$=$</td>
</tr>
<tr>
<td></td>
<td>MA-Pro/ret</td>
<td>0.103 $\pm$ 0.009</td>
<td>0.112 $\pm$ 0.011</td>
<td>$=$</td>
</tr>
<tr>
<td></td>
<td>MA-Ab/add</td>
<td>0.108 $\pm$ 0.007</td>
<td>0.189 $\pm$ 0.028</td>
<td>$+$</td>
</tr>
<tr>
<td></td>
<td>MA-Flex/Extend</td>
<td>0.139 $\pm$ 0.186</td>
<td>0.187 $\pm$ 0.022</td>
<td>$+$</td>
</tr>
<tr>
<td>CIF</td>
<td>PCSA</td>
<td>$6.93 \times 10^8 \pm 5.74 \times 10^{-6}$</td>
<td>$6.23 \times 10^8 \pm 9.93 \times 10^{-9}$</td>
<td>$=$</td>
</tr>
<tr>
<td></td>
<td>MA-Pro/ret</td>
<td>0.204 $\pm$ 1.43 $\times 10^{-6}$</td>
<td>0.317 $\pm$ 0.052</td>
<td>$+$</td>
</tr>
<tr>
<td></td>
<td>MA-Ab/add</td>
<td>0.077 $\pm$ 0.021</td>
<td>0.237 $\pm$ 0.039</td>
<td>$+$</td>
</tr>
</tbody>
</table>
Values reported are means ± SE (PCSA in m², MA is dimensionless, N = 6 cryptodires, 6 pleurodires). =, two lineages statistically equivalent; +, pleurodire mean greater than cryptodire (Cohen’s d >1). PIFI, puboischiofemoralis internus; ILF, iliofemoralis; FT, femorotibialis; FTI, flexor tibialis internus; CIF, caudi-iliofemoralis.
Table 2. Muscle activity patterns during locomotion while swimming and walking in cryptodire and pleurodire turtles.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Action</th>
<th>Swim</th>
<th>Walk</th>
<th>Canon 1</th>
<th>Canon 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cryptodire</td>
<td>Pleurodire</td>
<td>Cryptodire</td>
<td>Pleurodire</td>
</tr>
<tr>
<td>PIFI</td>
<td>On</td>
<td>43.08 ± 1.38</td>
<td>48.35 ± 0.45</td>
<td>53.12 ± 1.22</td>
<td>77.96 ± 0.73</td>
</tr>
<tr>
<td></td>
<td>Off</td>
<td>82.80 ± 0.83</td>
<td>89.23 ± 0.27</td>
<td>86.53 ± 1.01</td>
<td>101.76 ± 0.32</td>
</tr>
<tr>
<td>ILF</td>
<td>On</td>
<td>35.37 ± 1.21</td>
<td>55.18 ± 1.76</td>
<td>46.83 ± 1.20</td>
<td>76.57 ± 0.99</td>
</tr>
<tr>
<td></td>
<td>Off</td>
<td>65.09 ± 1.07</td>
<td>84.11 ± 1.52</td>
<td>74.83 ± 1.27</td>
<td>98.35 ± 0.47</td>
</tr>
<tr>
<td>FT</td>
<td>On</td>
<td>5.24 ± 0.97</td>
<td>31.87 ± 1.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>swing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Off</td>
<td>91.25 ± 0.65</td>
<td>104.02 ± 0.72</td>
<td>93.95 ± 0.60</td>
<td>99.62 ± 0.26</td>
</tr>
<tr>
<td>FTI</td>
<td>On</td>
<td>-3.38 ± 0.74</td>
<td>-0.96 ± 0.66</td>
<td>-0.71 ± 0.86</td>
<td>0.49 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>Off</td>
<td>22.29 ± 1.21</td>
<td>18.43 ± 1.13</td>
<td>28.50 ± 0.93</td>
<td>53.60 ± 1.16</td>
</tr>
<tr>
<td>CIF</td>
<td>On</td>
<td>-4.23 ± 0.73</td>
<td>1.82 ± 0.50</td>
<td>-0.14 ± 0.39</td>
<td>1.50 ± 0.60</td>
</tr>
<tr>
<td></td>
<td>Off</td>
<td>19.94 ± 1.26</td>
<td>22.27 ± 0.61</td>
<td>30.73 ± 0.89</td>
<td>47.35 ± 1.48</td>
</tr>
</tbody>
</table>

Values are mean activity timing (in percentage of limb cycle, 0 being a fully protracted limb) ± SE. Canonical values are loaded scores for each variable in the CDA. PIFI, puboischiofemoralis internus; ILF, iliofemoralis; FT, femorotibialis; FTI, flexor tibialis internus; CIF, caudi-iliofemoralis.
CHAPTER FOUR

VARIATION IN MORPHOLOGY AND KINEMATICS UNDERLIES VARIATION IN SWIMMING STABILITY AND TURNING PERFORMANCE IN FRESHWATER TURTLES

This is a pre-copy-editing, author-produced copy of an article in review for publication in the journal Integrative and Comparative Biology.

ABSTRACT

Among swimming animals, stable body designs often sacrifice performance in turning, and high turning performance may entail costs in stability. However, some rigid-bodied animals appear capable of both high stability and turning performance during swimming by propelling themselves with independently controlled structures that generate mutually opposing forces. Because such species have traditionally been studied in isolation, little is known about how variation within rigid-bodied designs might influence swimming performance. Turtles are a lineage of rigid-bodied animals, in which most species use contralateral limbs and mutually opposing forces to swim. We tested the stability and turning performance of two species of turtles, the pleurodire *Emydura subglobosa* and the cryptodire *Chrysemys picta*. *E. subglobosa* exhibited both greater stability and turning performance than *C. picta*, potentially through the use of subequally-sized (and larger) propulsive structures, faster limb movements, and decreased limb excursions. These data show how, within a given body design, combinations of different traits can serve as mechanisms to improve aspects of performance with competing functional demands.
INTRODUCTION

Animals exhibit extensive morphological and behavioral variation. Understanding the functional role of this variation can provide insight into the mechanisms that promote diversity in the performance and ecology of animals (Anderson and Patek, 2015; Wainwright and Price, 2016). In the locomotor system, one factor potentially contributing to variation is that many components of locomotor performance can have opposing requirements (Fish, 2002; Weihs, 2002; Webb, 2006). An example of such conditions can be found among aquatic animals through comparisons of hydrodynamic stability and turning performance.

Hydrodynamic stability is the resistance to, and recovery from, disturbances to an intended trajectory (Webb, 2006; Webb and Weihs, 2015), including those produced by recoil motions of the body during the movement of its propulsive structures (Bartol et al., 2003; Webb, 2002; Weihs, 2002). The movements of these structures can result in substantial perturbations, either laterally or dorsoventrally, depending on the way the propulsive structures are moved. Stability is quantified by the time it takes for the animal to recover from disturbances, as well as the translational and rotational deviations of an animal from a straight path (Full et al., 2002; Webb and Weihs, 2015). Such deviations can occur as lateral motions (sideslip and yaw), vertical motions (heave and pitch), or longitudinal motions (surge and roll). As the costs of steady swimming can represent most of the daily energy budget of swimming animals, stability is a critical component of aquatic locomotor performance, and failure to resist destabilizing forces could
significantly elevate energetic costs (Brett, 1995; Schultz and Webb, 2002; Webb and Weihs, 2015).

In contrast to stability, turning involves intentional changes of an animal’s trajectory and is commonly quantified using two distinct metrics: maneuverability and agility (Webb and Weihs, 2015). Manoeuverability is the ability to turn within a limited space and is reflected by the minimum radius (R) of the turning path (Howland, 1974; Norberg and Rayner, 1987; Walker, 2000). Agility is the rate of turning, which is measured as the maximum angular velocity ($\omega_{\text{max}}$) exhibited during a turn (Norberg and Rayner, 1987; Webb, 1994). Superior turning performance can, therefore, be characterized by an animal possessing high maneuverability (low R) and high agility (high $\omega_{\text{max}}$). Like stability, turning is a critical aspect of swimming performance that plays an important role in capturing prey, eluding predators, and navigating complex habitats (Domenici, 2001; Domenici et al., 2008; Garland and Losos, 1994; Maie et al., 2014; Webb, 1983; Weihs, 1972; Weihs, 1993).

Both stability and turning can impact an animal’s fitness (Higham et al., 2016). However, the demands of these aspects of locomotor performance can be at odds (Fish, 2002; Webb, 2006; Weihs, 2002). Body designs that are stable typically require substantial forces to change course; as a result, stable designs can impede turning, making turns more difficult or costly to execute. (Weihs, 2002). Because of these opposing demands, many animals exhibit morphological and behavioral traits that help to improve either stability (Bartol et al., 2003; Bartol et al., 2005; Rivera et al., 2011; Sefati et al., 2013), or turning performance (Calsbeek and Irschick, 2007; Garland and Losos,
1994). These can include differences in body shape, propulsor and control surface
morphology and position, and body rigidity. For example, a rigid body might improve
stability, but can directly decrease maneuverability by inhibiting longitudinal bending,
and decrease agility by reducing the second moment of area about the dorsoventral
rotational axis (Walker, 2000). Therefore, animals with rigid bodies are commonly stable
but show low turning performance (Fish and Nicastro, 2003). As rigidity decreases,
turning ability typically improves. For example, animals with stiff (rather than rigid)
bodies, such as tuna and many cetaceans, typically have intermediate turning
performance (Blake et al., 1995; Fish, 2002), and flexible animals usually have high
turning performance (Domenici and Blake, 1997).

Despite these patterns, some animals with rigid bodies overcome low turning
performance by using mutually opposing (antagonistic) forces during locomotion
(Walker, 2000; Fish and Nicastro, 2003; Rivera et al., 2006; Sefati et al., 2013; Jastrebsky
et al., 2016). In these animals, propulsive structures are used in orthogonal pairs that beat
out of phase to balance forces and torques (Hove et al., 2001). The control of these
structures enhances dynamic stability during linear swimming as well as turning
performance during unsteady behaviors (Bartol et al., 2008; Sefati et al., 2013; Webb and
Weihs, 2015). However, such evaluations of performance have typically been based on
comparisons of rigid-bodied taxa to flexible or stiff-bodied lineages. Thus, there is
limited understanding of how morphological and behavioral variation influence
hydrodynamic stability and turning performance within particular body designs.
Turtles are a lineage of rigid-bodied animals with a unique body plan that facilitates testing the factors that influence performance in hydrodynamic stability and turning. Because most of the vertebral column is fused dorsally with a bony carapace (Walker, 1973), all propulsive thrust must be produced by the four limbs (Pace et al., 2001). This simplification of the locomotor system, and their use of only two pairs of limbs for locomotion as opposed to rigid-bodied fishes that use multiple fins and fin types to swim (5 fins, 4 types), makes interspecific comparisons of turtle performance extremely tractable. Turtles generally swim using one of two strategies: dorsoventral flapping or anteroposterior rowing. Sea turtles use dorsoventral flapping of the forelimbs during swimming, and experience substantial heave and pitch during swimming (Dougherty et al., 2010; Rivera et al., 2011). In contrast, nearly all freshwater turtle species use rowing to swim, in which synchronous movements of the oar-like contralateral fore-and hindlimbs occur primarily in an anteroposterior plane (Blob et al., 2008; Rivera et al., 2010; Mayerl et al., 2017). These patterns have been suggested to enhance stability by minimizing yaw (lateral rotations around the dorsoventral axis of the body) (Zug, 1971; Rivera et al., 2011), with any yawing motions that persist caused by differences in the direction and magnitude of thrust production between a given pair of contralateral forelimb and hindlimb (Rivera et al., 2011).

Although comparisons of swimming performance between flapping and rowing are well established (Davenport et al., 1984; Rivera et al., 2011), very little is known about how morphological and behavioral differences within freshwater turtles may impact swimming performance (Blob et al., 2008; Young et al., 2017). There is extensive
variation in freshwater turtles in both the extent of webbing of the manus and pes, and patterns of limb movement (Pace et al., 2001; Bonin et al., 2006, Rivera et al., 2011, 2013; Mayerl et al., 2016, 2017; Young et al., 2017). As webbing between the digits contributes directly to the surface area of a propulsive element, it is also expected to correlate with the propulsive forces produced by the appendages. Thus, differences in webbing between the manus and pes should increase differences in anterior and posterior force production and, thereby, elevate destabilizing torques during swimming (Rivera et al., 2011). In contrast, more equal webbing between the forelimbs and hindlimbs would be expected to reduce unintended destabilizing torques and, thus, facilitate swimming with a more stable trajectory. Variation in kinematic patterns might also lead to variation in stability and turning performance. Large anteroposterior limb excursions would likely produce high levels of laterally directed thrust and, thus, increase recoil motions of the body, whereas highly aquatic species that limit their limb excursions may be more stable (Blob et al., 2008). Smaller limb excursions might also decrease the ability of turtles to change direction in turns, but few data are available from turtles to test this prediction (Rivera et al., 2006).

The goals of this study were to test how variation in morphology and kinematics impact stability and turning performance in turtles. We measured stability and turning performance in two freshwater species: the Pink-bellied sideneck turtle, *Emydura subglobosa* (Krefft 1876), and the Painted turtle, *Chrysemys picta* (Schneider 1783). These two species of turtle both spend most of their time in the water and share an aquatic common ancestor. However, *E. subglobosa* is a member of the pleurodire lineage,
all of which are primarily aquatic, whereas *C. picta* belongs to the cryptodire lineage, which includes many semi-aquatic and terrestrial taxa (Joyce and Gauthier, 2004). Several factors suggest that *E. subglobosa* may have greater swimming stability than *C. picta*. For example, pleurodires have a pelvic girdle that is fused to the shell. This leads to smaller rotations of the pelvic girdle during locomotion than in cryptodires, which could increase stability (Mayerl et al., 2016). Qualitative observations also indicate that *E. subglobosa* has more extensive webbing in the manus than *C. picta*, potentially similar to the extent of webbing in its hindfeet. Similarity in the distribution of webbing between the manus and pes in *E. subglobosa* could facilitate more equal thrust production from fore- and hindlimbs that would increase swimming stability relative to *C. picta*. Previous research on the swimming kinematics of these two species also indicates that limb excursions of *E. subglobosa* are likely smaller than those of *C. picta* (Rivera et al., 2011; Mayerl and Blob, 2017). Smaller limb excursions in *E. subglobosa* might also contribute to increased stability compared to *C. picta* by reducing the amount of lateral thrust production that would occur at the beginning and end of each limb cycle. In contrast to predictions for differences in stability between these taxa, expectations for differences in their turning performance are less clear. Because high stability usually comes at a cost to high turning performance, it is possible that *C. picta* will have higher turning performance than *E. subglobosa*. However, the use of mutually opposing propulsive forces might allow *E. subglobosa* to retain strong turning performance despite high stability.
We expect that *E. subglobosa* will be more stable than *C. picta* due to a combination of morphological (more similar sized fore- and hindlimbs) and behavioral (decreased kinematic excursions) features, and test turning performance in both species to evaluate if increased stability in turtles comes at a cost to turning performance. Through these evaluations of how morphology and kinematic behavior relate to aquatic locomotor performance, we can gain insight into factors that allow animals to meet multiple functional demands, and broaden the foundation for building computational models of swimming performance.

**MATERIALS AND METHODS**

*Experimental animals*

Four adult male *E. subglobosa* (16.5-18.4 cm) were purchased from a commercial supplier (Turtles and Tortoises, Inc., Brooksville, FL). Four adult male *C. picta* (10.1-13.4 cm), were collected from a spillway of Lake Hartwell, Pickens County, SC, USA (South Carolina Scientific Collection permit # 28 – 2016). Turtles were housed in pairs in a temperature controlled greenhouse facility, using 600-liter stock tanks equipped with dry basking platforms, a submerged 200-watt heater (maintaining water at 25 deg C) and pond filters. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Clemson University (protocol 2015-033).

*Measurement of swimming performance and kinematics*
To collect data on swimming performance, we trained turtles to swim to chase a prey stimulus at maximal velocity, which was pulled through the water either in a straight line (stability trials, *E. subglobosa*: 108 cycles, *C. picta*: 84 cycles) or through a path in which that same straight line was followed by a 90 deg turn (turning performance trials, *E. subglobosa*: 45 turns, *C. picta*: 43 turns). All turtles performed both stability and turning performance trials. There is slight variation in the number of trials per turtle due to differences in the ability to train each turtle to follow the prey stimulus, and to maintain training. The movement path of the prey stimulus was controlled by a custom-built robotic gantry with two degrees of freedom, which motivated turtles to swim in comparable paths across trials (i.e., reproducible straight lines, turn angles, and velocities). Due to slight differences in swimming speed across our turtles, the velocity of the path was set for each turtle to ensure that the stimulus did not go so fast that the turtle would lose interest in chasing, or so slow that the turtle would capture the stimulus prior to the conclusion of the trial. During trials, kinematic data were collected at 100 Hz using two digitally synchronized, high-speed video cameras (Phantom V5.1, Vision Research, Inc.; Wayne, NJ, USA) in lateral and ventral views, with the ventral view obtained via a mirror angled at 45 deg with respect to the transparent bottom of the tank. We collected three full locomotor cycles per stability trial, with each single cycle defined as beginning when the left forelimb was fully protracted, and ending at the next point of full forelimb protraction in the same limb (N = ~ 10 trials, resulting in 30 strokes per turtle). We collected data from a single turn in each turning trial (N = ~15 turns per turtle), with each turn taking 1.5 cycles of the left (outer) forelimb (where the limb starts fully protracted, is
retracted, then protracted, and finishes in a retracted state). This cutoff was assigned because all turtles completed turns and resumed anteroposterior movements of the limbs that are characteristic of linear swimming within this period.

To facilitate kinematic and performance measurements from videos, turtles were marked with high-contrast, trackable points (non-toxic, white nail polish with a central, black point; Fig. 1). Limb protraction and retraction angles, and lateral stability metrics (sideslip and yaw) were calculated from body landmarks in the ventral view (i.e., nose, anterior and posterior plastron, shoulders, hips, elbows, and knees). Vertical stability metrics (heave and pitch) were calculated from body landmarks in the lateral view (i.e., nose and anterior and posterior tips of the carapace). Marked points were tracked using DLTDataViewer5 (Hedrick, 2008), and the resulting two-dimensional coordinate data were processed using custom MatLab routines to determine the center of rotation (COR) of the turtle for each view, and to calculate kinematic and stability metrics (Rivera et al., 2011). To facilitate comparisons of kinematic and stability profiles for locomotor cycles of different absolute durations, a quintic spline was applied to smooth and interpolate the data to 101 values, representing 0 – 100% of the limb cycle (0 and 100% being a fully protracted left forelimb) (Walker, 1998). We calculated the stride duration for each limb cycle and used that in conjunction with the angular excursion of the limb to calculate limb excursion angular velocity.

To analyze turning performance, the COR for each turn was processed through QuicKurve (Walker, 2000), which fits a quintic spline through the x-y coordinates of the COR and calculates the minimum instantaneous radius of the turn (R) (Walker, 2000;
Rivera et al., 2011). To facilitate comparisons of turning radius between animals of different sizes, we standardized R by carapace length (Walker, 2000; Jastrebsky et al., 2016). We also calculated the space required to make a turn (R_{space}), which incorporates the width of the body to estimate the amount of space an animal needs to execute a turn (Walker, 2000). Turning rate (\omega) was calculated from the splined x-y coordinate data using custom Matlab routines to calculate the first derivative of the angle of the turn throughout a turn. We isolated the maximum turning rate for each turn to determine the maximum instantaneous turning rate (\omega_{max}), and also calculated the average instantaneous turning rate throughout a turn (\omega_{avg}). We also calculated how long it took for each turn to be completed by documenting when the turtles resumed linear swimming kinematics.

**Collection of morphometric data**

To test for differences in the propulsive morphology of our two focal species, we collected linear measurements from our experimental animals including lengths of the brachium, antebrachium and manus (forelimb), and thigh, crus and pes (hindlimb). To increase our sample for morphometric analyses we supplemented the data from our experimental animals with data from freshly frozen specimens (total sample: N = 6 *E. subglobosa*, N = 7 *C. picta*). Photographs of the posterior portions of the left forelimb and hindlimb were collected and analyzed using ImageJ (Schneider et al., 2012), allowing calculations of the area of each element of the limb through which we could compare estimates of their relative propulsive contributions between species.
**Statistical analyses**

All statistical analyses were performed in R (V 3.2.1, www.r-project.org). We used linear mixed effects models to evaluate potential differences between species in stability and turning performance (lmer4; Bates et al., 2015). For stability trials, we used species as a fixed effect, with individual and trial (intercept varying within trial) as random effects (X ~ species*velocity + 1|individual/trial). For turning performance trials, we used species as a fixed effect, with individual as a random effect. P-values were obtained by likelihood ratio tests of the full model with the effect in question. Effect sizes based on mixed effects models were calculated following published methods (Cohen, 1992). We evaluated differences in limb angular excursion (protraction/retraction) between species for linear swimming using principal components analyses (prcomp; R Core Team, https://www.R-project.org), and for turning using discriminant analyses (lda; R Core Team, https://www.R-project.org). Differences in limb area were evaluated using Cohen’s D (effsize; R package version 0.7.0, https://CRAN.R-project.org/package=effsize). All data are presented as mean ± standard error unless otherwise noted.

**RESULTS**

*Swimming performance*

Velocity was similar between the two species during linear swimming (*E. subglobosa* = 2.69 BL sec\(^{-1}\) ± 0.65 s.d., *C. picta* = 2.88 BL sec\(^{-1}\) ± 0.57 s.d.; *p* = 0.06, D = 0.34). The position of the center of rotation along the anteroposterior body axis was also similar
between the two species during linear swimming (\textit{E. subglobosa} = 40.42 % ± 1.11, \textit{C. picta} = 38.79 % ± 1.66 SE; p = 0.77, D = 0.12). However, we found that \textit{E. subglobosa} was between 30\% and 40\% more stable for all measures of stability than \textit{C. picta} (all D > 0.75, Fig. 2, table S1). This was especially apparent for yaw, as \textit{E. subglobosa} experienced almost half the yaw excursion throughout a given limb cycle than \textit{C. picta} (\textit{E. subglobosa}: 7.39 ± 0.319; \textit{C. picta}: 12.32 ± 0.385, p < 0.001, D = 1.68).

\textit{E. subglobosa} also had better turning performance than \textit{C. picta} (Fig. 3, table S1). The mean of the minimum instantaneous turning radius of \textit{E. subglobosa} was approximately half that of \textit{C. picta}, and the overall 90\th percentile best turn of \textit{E. subglobosa} was an order of magnitude better than that of \textit{C. picta} (\textit{E. subglobosa}: \textit{R}_{90} = 0.008 BL, \textit{C. picta}: \textit{R}_{90} = 0.083 BL, Fig. 3, table S1). \textit{E. subglobosa} also used a smaller turning path throughout its turn than \textit{C. picta} (\textit{E. subglobosa}: \textit{R}_{\text{space}} = 0.283 ± 0.044 SE BL, \textit{C. picta}: \textit{R}_{\text{space}} = 0.436 ± 0.044 SE BL, Table S1). Similarly, mean maximum turning rate (\textit{\omega_{\text{max}}}) and the 90\th percentile fastest turns were much faster (~100 deg sec\textsuperscript{-1}) in \textit{E. subglobosa} than \textit{C. picta} (\textit{E. subglobosa}: \textit{\omega_{\text{max}}} = 383.21 ± 9.83 deg sec\textsuperscript{-1}, \textit{\omega_{\text{max}-90}} = 477.80 deg sec\textsuperscript{-1}, \textit{C. picta}: \textit{\omega_{\text{max}}} = 310.56 ± 34.00 deg sec\textsuperscript{-1}, \textit{\omega_{\text{max}-90}} = 397.46 deg sec\textsuperscript{-1}, Fig. 3, table S1). Average turning rate throughout a turn was faster in \textit{E. subglobosa} than in \textit{C. picta} (\textit{E. subglobosa}: \textit{\omega_{\text{avg}}} = 229.78 ± 8.58 deg sec\textsuperscript{-1}, \textit{C. picta}: \textit{\omega_{\text{avg}}} = 174.44 ± 8.70 deg sec\textsuperscript{-1}, Table S1).

\textit{Kinematics}
During linear swimming, both species of turtle swam using typical asynchronous rowing, in which one set of contralateral limbs was protracted while the other set was being retracted (Movie 1; Fig. S1A, C). Principal components analyses show that kinematic excursions of the limbs differed between the species during linear swimming (Fig. 4; table S2), as *C. picta* retracted and protracted their fore- and hindlimbs more than *E. subglobosa*, resulting in a greater angular excursion of the limbs (Fig. 4A; Fig. S1, table S2). Although stride duration was shorter in *E. subglobosa* (*E. subglobosa*: 0.257 ± 0.012 sec, *C. picta*: 0.327 ± 0.014 sec; *p* < 0.001, *D* = 1.15), the smaller angular excursions of the limbs resulted in the two species moving their limbs at the same velocity (Fig. 5, Table S3).

During turns, the outside (left) limbs were used in a similar fashion to the limbs during linear swimming, but the inside two limbs were used as a rudder, or brake, as they were maintained at a stable angle throughout much of the duration of the turn (Fig. S1 B, D; Movie 2). Linear discriminant analysis showed that the primary kinematic variables that differentiated the two species were that pleurodires protracted their outside (left) arm less, protracted their outside leg more, and retracted their inside (right) leg less (Fig. 4B, Table S4). *E. subglobosa* completed the turning maneuver in relatively less time than *C. picta* (*E. subglobosa*: ~1.1 strokes of the outer forelimb, 0.317 ± 0.019 s; *C. picta*: 1.5 strokes, 0.394 ± 0.019 s, *p* = 0.006, *D* = 1.232, Fig. S1B, D). This resulted in *E. subglobosa* resuming use of their inside (right) forelimb for propulsion in less than the 1.5 cycles that we defined as a turn, whereas *C. picta* held the inside limb stationary at approximately 45 deg to the long axis of the shell throughout the duration of the turn.
(Fig. S1B; table S4). During turns, limb velocity of both forelimbs and the right hindlimb were similar between the two species, although *E. subglobosa* moved its left (outer) hindlimb at a greater velocity than *C. picta* (Fig. 5, Table S5).

*Morphology*

Both species exhibited relatively similar overall arm and leg lengths when standardized by body length (table 1). We found that *E. subglobosa* had much larger areas for their distal limb elements than *C. picta* (table 1, Fig. S2). Furthermore, the manus and pes were more similar in size to each other in *E. subglobosa* than in *C. picta*, which had much smaller forelimbs than hindlimbs (table 1, Fig. S2).

**DISCUSSION**

*Freshwater turtle stability and turning performance compared with other rigid-bodied taxa*

Broadly, both species of turtle show stability and turning performance that are similar to other rigid-bodied swimmers that use multiple propulsors to move. Such rigid-bodied swimmers typically demonstrate high stability and maneuverability, but low agility when compared with flexible-bodied taxa (Gerstner, 1999; Walker, 2000; Hove et al., 2001; Fish and Nicastro, 2003; Wiktorowicz et al., 2007; Jastrebsky et al., 2016). However, most previous work on the stability and turning performance of rigid-bodied taxa has compared distantly related lineages (Walker, 2000; Hove et al., 2001; Bartol et al., 2003; Fish and Nicastro, 2003; Rivera et al., 2006; Rivera et al., 2011). Our focus on relatively
closely related species in this study can refine insight into potential mechanisms that
govern aquatic locomotor performance, because it reduces the range of differences across
numerous physiological and structural variables that can impact performance. We found
that the pleurodire turtle *E. subglobosa* exhibited both higher stability and better turning
performance than the cryptodire *C. picta*. *E. subglobosa* was also able to recover from a
turn and begin swimming straight faster than *C. picta*, suggesting that it is more able to
recover from disturbances as well as resist them. These insights can facilitate exploration
of structures and behaviors that might help animals to improve both stability and turning
performance, despite their potentially competing demands.

**Mechanisms contributing to variation in stability**

A combination of both morphological and behavioral features may enable *E.
subglobosa* to exhibit greater stability than *C. picta*. We expected that greater equality of
manus and pes sizes, as well as smaller kinematic excursions of the limbs, would enhance
stability. Our results largely agree with our predictions. *E. subglobosa*, the more stable
species, had forelimbs that were much closer in size to its hindlimbs than *C. picta* (Table
1). Having limbs of similar areas would enhance linear swimming stability by promoting
the potential for rotational forces generated by contralateral fore- and hindlimbs to offset
one another more effectively (Sefati et al., 2013). This would subsequently reduce yaw
and sideslip in *E. subglobosa*, as these two metrics are often correlated (Rivera et al.
2011). *E. subglobosa* was also more stable in the vertical stability parameters of pitch and
heave. No a priori hypotheses were established for these parameters; however, the
differences we observed could potentially be due to differences in the amount of
dorsoventral movement of the limbs in *E. subglobosa* and *C. picta*. There are limits to the
conclusions that can be drawn from our analyses with regard to how kinematics influence
force production, because our kinematic data are two-dimensional, do not include the
distal portions of the limbs, and we have not directly measured forces. However, the
alignment of our predictions with differences in measured swimming performance
between our focus species suggests that both subequal propulsor sizes and limited
kinematic excursion of the limbs during linear swimming could play a substantial role in
increasing swimming stability.

*Mechanisms contributing to variation in turning performance*

Although many broad comparisons have found that more stable species have lower
turning performance, we found that *E. subglobosa* had higher turning performance in
addition to being more stable than *C. picta*. Differences in morphology and kinematic
behavior also may contribute to these results. The larger manus and pes of *E. subglobosa*
provide larger control surfaces, which could increase the thrust generated by the outer
limbs as well as increase the braking potential of the inner limbs during turns (Fish and
Lauder, 2017). *E. subglobosa* also used kinematic excursions of its outer legs that were as
large as those of *C. picta* when turning (Fig. S1B, D, table S4). This suggests that the
small limb excursions used by *E. subglobosa* during linear swimming were likely
produced through behavioral plasticity, rather than anatomical constraints that we had
predicted to be imposed by restrictions on pelvic girdle movement in pleurodires (Mayerl
et al., 2016). Furthermore, because intentional turning is, by definition, an unsteady behavior, the increased excursion of the limbs to facilitate turns in _E. subglobosa_ suggests that limiting the excursion of the limbs during linear swimming may enhance the stability of this species. Another factor likely contributing to the better turning performance of _E. subglobosa_ is the greater velocity of its propulsive limbs during turns, as _E. subglobosa_ moved its outer, thrust generating hindlimb faster during turns than _C. picta_ (_E. subglobosa_: 368.74 ± 15.20 deg sec\(^{-1}\), _C. picta_ 270.31 ± 15.5 deg sec\(^{-1}\), Table S5).

Correlations of performance variation with differences in structure and habitat between turtle lineages
The differences in swimming performance we observed between _E. subglobosa_ and _C. picta_ may relate to differences in the typical preferred habitats of these species. Although both of these species can walk on land, there are no fully terrestrial pleurodire taxa, whereas close relatives of _C. picta_ are semi-aquatic or even fully terrestrial (e.g., box turtles from the genus _Terrapene_: Bonin et al., 2006; Ernst and Lovitch, 2009). Pleurodires, as a lineage, may generally be more specialized for aquatic habitats than most rowing cryptodires. Cryptodires that spend more time on land may be subject to other functional demands that limit specialization for aquatic performance. Tradeoffs in locomotor performance between water and land are well documented, and future research could explore this topic in turtles (Shine and Shetty, 2001; Isaac and Gregory, 2007; Blob et al. 2016).
Other differences in the life history and habits of these species might also place a relative premium on aquatic performance in *E. subglobosa*. *E. subglobosa* are carnivorous throughout their entire lives, whereas *C. picta* are omnivores (Cann, 1998; Bonin et al., 2006; Ernst and Lovitch, 2009). Higher performance in both stability and turning might be advantageous for *E. subglobosa* during episodes of chasing prey, but less critical for *C. picta* with a diet consisting partly of plants and scavenged material.

The extent to which such ecological and evolutionary differences between these taxa have shaped their differences in swimming performance is difficult to evaluate without broader phylogenetic sampling that could establish the evolutionary history of these traits (Garland and Adolf, 1994). Performance comparisons across additional, diverse taxa and in different environmental conditions could further clarify the roles of adaptation, constraint, and tradeoffs in shaping aquatic locomotor performance.

**Conclusions**

Our study highlights the importance of investigating how variation within a given body design impacts performance. *E. subglobosa* demonstrated both higher stability and greater turning performance than *C. picta*. These differences in performance likely arise through a combination of variation in both morphology (having larger areas of propulsive structures), and behavior (differences in limb excursion, and in limb velocity during turns). The potential for freshwater turtles to use pairs of contralateral limbs to generate mutually opposing perturbating forces may allow species to achieve both high stability.
and high turning performance by behaviorally modulating their propulsors (Sefati et al., 2013). Investigating the interplay between morphology and behavior may ultimately inspire design features for human-engineered vehicles, which have traditionally been unable to achieve both high stability and high maneuverability (Jing and Kanso, 2013; Webb and Weihs, 2015). The design and behavior features present in turtles that enable one species to have superior performance than others could provide a model for biomimetic designs of autonomous underwater vehicles that combine superior stability with an ability to execute rapid turns in a limited space. Turtles also represent a system rich with potential for future study into the evolution of performance in different environments, as they have maintained a similar body shape throughout their evolutionary history, but have diversified to occupy nearly all water bodies around the globe (Bonin et al., 2006).
ACKNOWLEDGEMENTS

We would like to thank A. Arellanez, J. Pruett, K. Smith, L. Stevens, C. Petty, and A. Sansone for their assistance collecting and processing data, and M. Sears for his assistance with statistics.

FUNDING

This work was supported by a Society for Integrative and Comparative Biology Grant-in-aid-of-research to C.J.M., and a Clemson University Creative Inquiry Grant (#479) to R.W.B.
REFERENCES


Cambridge: Cambridge University Press.


**FIGURES AND TABLES**

**Figure 1.** Anatomical landmarks used to track limb and body movements for evaluation of stability and turning performance during swimming. (A) Lateral view: a – nose, b – anterior carapace, c – posterior carapace, (B) ventral view: 1 – nose, 2 – anterior plastron, 3 – posterior plastron, 4 – right shoulder, 5 – right elbow, 6 – left shoulder, 7 – left elbow, 8 – right hip, 9 – right knee, 10 – left hip, 11 – left knee.
Figure 2. Violin plots comparing the stability (in excursion) of *E. subglobosa* (pleurodire) and *C. picta* (cryptodire) during linear swimming in (A) heave, (B) pitch, (C) sideslip, and (D) yaw. Large black circles indicate means, and small black circles indicate outliers. The width of the violin plot represents the distribution of the data along the y-axis. *E. subglobosa* was more stable (i.e., had lower parameter values) in all four metrics of stability. *, Significant difference between groups (p <0.05).
Figure 3. Violin plots comparing turning performance between *E. subglobosa* (pleurodire) and *C. picta* (cryptodire). (A) Minimum instantaneous turning radius (in body lengths) for each species. (B) Maximum turning rate (deg sec$^{-1}$) of each species. Large black circles indicate mean, white diamonds indicate 90% best performance, and small black circles indicate outliers. The width of the violin plot represents the distribution of the data along the y-axis. The pleurodire had a smaller turning radius and higher turning rate than the cryptodire, indicating superior turning performance. *, Significant difference between groups.
Figure 4. Multivariate kinematic results during linear swimming (A) and turning (B).

Green: *E. subglobosa* (pleurodire); blue: *C. picta* (cryptodire). Ellipses in (A) indicate one standard deviation from the mean for each species. Focal turtle species used distinct kinematics while swimming along straight paths. Larm: left arm; Rarm: right arm; Lleg: left leg; Rleg right leg. Min: maximal protraction angle of the limb; Max: maximum retraction angle of the limb. (B) Violin plot of the first linear discriminant, where the left limbs were the outer limbs. Large black circles indicate the mean, small black dots indicate outliers, an asterisk indicates a statistically significant difference between the two groups. Differences between *E. subglobosa* and *C. picta* are defined primarily by decreased protraction of the left forelimb, increased protraction of the left hindlimb, and decreased retraction of the right hindlimb in *E. subglobosa* relative to *C. picta* (Table S4).
**Figure 5.** Violin plots comparing mean limb velocity for *E. subglobosa* (green) and *C. picta* (blue). Format follows Figures 2 and 3. (A) Forelimb velocity during linear swimming; (B) hindlimb velocity during linear swimming; (C) left forelimb velocity during turning; (D) left hindlimb velocity during turning; (E) right forelimb velocity during turning; (F) right hindlimb velocity during turning.
Table 1. Comparison of standardized limb lengths and areas (measurements divided by carapace length and carapace length squared, respectively) between *E. subglobosa* (pleurodire, N = 6) and *C. picta* (cryptodire, N = 7) turtles.

<table>
<thead>
<tr>
<th>Variable</th>
<th><em>E. subglobosa</em></th>
<th><em>C. picta</em></th>
<th>P</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm length</td>
<td>0.40 ± 0.01</td>
<td>0.384 ± 0.01</td>
<td>0.2</td>
<td>0.77</td>
</tr>
<tr>
<td>Antebrachium area</td>
<td>0.011 ± 0.0005</td>
<td>0.010 ± 0.0006</td>
<td>.024</td>
<td>0.71</td>
</tr>
<tr>
<td>Manus area</td>
<td>0.016 ± 0.001</td>
<td>0.007 ± 0.0004</td>
<td>&lt;0.001</td>
<td>5.41</td>
</tr>
<tr>
<td>Leg length</td>
<td>0.519 ± 0.01</td>
<td>0.468 ± 0.02</td>
<td>0.04</td>
<td>1.19</td>
</tr>
<tr>
<td>Crus area</td>
<td>0.020 ± 0.0008</td>
<td>0.012 ± 0.0004</td>
<td>&lt;0.001</td>
<td>6.04</td>
</tr>
<tr>
<td>Pes area</td>
<td>0.025 ± 0.0009</td>
<td>0.016 ± 0.0009</td>
<td>&lt;0.001</td>
<td>3.96</td>
</tr>
<tr>
<td>Manus/Pes area</td>
<td>0.654 ± 0.032</td>
<td>0.456 ± 0.028</td>
<td>&lt;0.001</td>
<td>2.78</td>
</tr>
</tbody>
</table>
CHAPTER FIVE

BONE LOADING MECHANICS DURING LOCOMOTION IN PLEURODIRE TURTLES

ABSTRACT

A key feature that enable tetrapods to move through the environment is the ability of the skeleton to resist and transfer the loads that locomotion imposes on them. Some general patterns of limb bone loading have emerged from these analyses: parasagittal animals experience primarily bending and have lower safety factors, whereas sprawling animals experience bending as well as twisting, but have higher safety factors. However, few studies have examined how variation within a given lineage impacts bone loading mechanics. Here, we investigate how pelvic girdle fusion in pleurodire turtles may have influenced their bone loading mechanics relative to cryptodire turtles with an unfused, ancestral pelvic girdle. We found that safety factors between the two lineages were similar, but that the orientation of the loads that they experience were different. Understanding the mechanisms that drive such variation in loading mechanics within a given lineage could improve predictions about patterns between lineages.
INTRODUCTION

Locomotion through the environment is a critical activity for most vertebrates. In tetrapods, locomotion is typically supported by a robust limb skeleton that resists and transfers mechanical loads (Currey, 1984; Currey, 2002). Locomotion produces some of the most frequent and severe loads that the limbs must bear (Biewener, 1990; Biewener, 1993), and the capacity of limb bones to bear such loads may have served as a key feature in the initial radiation of tetrapods into terrestrial habitats (Kawano and Blob, 2013).

However, there is extensive variation within tetrapods in the morphology of the musculoskeletal system used for locomotion, as well as in the locomotor behaviors in which the musculoskeletal system is employed (Alexander, 1983; Blob, 2000; Currey, 1984; Currey, 2002; Erickson et al., 2002; Wilson et al., 2009). Testing the mechanical variation associated with such structural variation could be key in understanding how changes in mechanical loads on the musculoskeletal system may have contributed to the ecological and evolutionary history of a lineage.

*In vivo* measurements of bone strain are one common means of evaluating how the skeleton is loaded during locomotion (Biewener, 2002). These techniques allow for direct measurement of the loads experienced by bones during locomotion and, when coupled with measurements of the mechanical properties of bone tissues, can indicate the maximal loads the bones can experience without failure (Alexander, 1981; Alexander, 1997; Alexander, 1998). Framed as a ratio between the load that causes a structure to fail compared to the usual load that it experiences, this parameter is commonly called a safety
factor, and has been used extensively in engineered and biological structures (Alexander, 1981).

Safety factors are typically evaluated in the context of a specific loading regime that a bone experiences, such as bending or torsion (Butcher et al., 2008). The loading regime that a bone experiences during locomotion is dependent primarily on differences in posture and behavior during locomotion. Generally, running mammals experience limb bone loads dominated by bending patterns with safety factors ranging from two to four (Biewener, 1983; Biewener, 1993; Biewener et al., 1983; Blob et al., 2014). This is thought to be primarily due to their parasagittal posture, in which the ground reaction force (GRF) is aligned fairly parallel to the long axis of the limb bones (Biewener, 1989). In contrast, sprawling animals such as reptiles have been found to have higher safety factors of up to 10, and loading regimes that include both bending and high levels of shear strain that reflect twisting, or torsion (Blob et al., 2014). The increased amount of torsion that these animals experience is thought to be due to the GRF applying a torsional moment on the bone. Unlike with posture, the loading regime of a bone does not change with ontogeny or with changes in velocity, although the magnitude of strain does increase at higher speeds and body sizes (Main, 2007; Main and Biewener, 2006). Between distantly related species with disparate morphologies and within individual species, higher strain rates are also correlated with higher magnitude strains (Aiello et al., 2015; Butcher et al., 2008). However, despite the recognition of many of these broad patterns, there is limited understanding of how differences in morphology or patterns of motion across closely related species might correspond to different patterns of loading. In
instances where this has been evaluated, morphological and behavioral variation between closely related species is correlated with changes in bone strain magnitudes. For example, the sequential, short hops of toads are correlated with lower limb bone strains than the explosive jumps of bullfrogs (Blob et al., 2014). However, other instances of such comparisons of bone loading are generally lacking, making the extent to which within-lineage differences in loading patterns might scale to across-lineage differences unclear.

Turtles represent an outstanding system for examining how variation in limb structure correlates with variation in loading patterns and magnitudes during locomotion. Due to the fusion of the vertebral column with the dorsal carapace, all thrust for locomotion in turtles is derived from the limbs (Pace et al., 2001), which limits confounding effects related to differences in the incorporation of other body structures in propulsion in comparisons across species. In particular, this vertebral fusion eliminates the potential for lateral bending of the body axis, a factor that has been proposed to explain why turtles experience the highest torsional loads measured for the limb bones of walking vertebrates (Butcher et al., 2008). These high twisting loads are especially relevant in the context of evaluating safety factors, as bone is much more likely to fail under torsional loads than it is when loaded in bending or compression (Vogel, 2013). Moreover, though the general body plan of turtles has remained fairly constant throughout their evolutionary history (Bonin et al., 2006), there is variation in the structure of the locomotor system across turtle lineages that might impact bone loading mechanics during terrestrial locomotion.
There are two main lineages of turtles: cryptodires and pleurodires. Although the ancestor of all turtles was probably aquatic (Joyce and Gauthier, 2004), cryptodires have radiated onto land multiple independent times throughout their evolutionary history (e.g. box turtles, tortoises, Asian box turtles), whereas pleurodire lineages have remained primarily aquatic (Bonin et al., 2006). Among other morphological and molecular traits, one defining feature of these clades is the novel fusion of the pelvis to the shell in pleurodire turtles (Walker, 1973). Pelvic girdle fusion in pleurodires has resulted in changes in muscle leverage and use, especially during walking (Mayerl et al., 2017). This morphological feature has also resulted in differences in pelvic girdle mobility during locomotion. The pelvis of pleurodires does not rotate in conjunction with the stride, whereas the ancestral cryptodire pelvis rotates approximately 20 degrees per step (Mayerl et al., 2016). These differences in pelvic girdle mobility are also correlated with changes to femoral kinematics, as pleurodires exhibit greater long axis rotation of the femur and decreased stride lengths compared to cryptodires (Mayerl et al., 2016).

The mechanical implications of the differences in pelvic girdle morphology between pleurodire and cryptodire turtles provide an excellent context for evaluating how changes in morphology can affect changes in bone loading regime and magnitude. Because all turtles are unable to use rotations of the body axis to reduce femoral twisting loads (Butcher et al., 2008), the fusion of the pelvis to the shell in pleurodire turtles may further increase the torsion that the pleurodire femur experiences during locomotion over that experienced by cryptodires. In this study, we collected in vivo bone strain data from the pleurodire Pelusios niger (Duméril and Bibron 1835) during terrestrial locomotion,
and compared these with previously collected data (Butcher et al., 2008) from the cryptodire *Pseudemys concinna* (Leconte 1830). These two species are both large, semiaquatic freshwater turtles, making them excellent for comparisons of bone loading. We predicted that pelvic girdle fusion would result in *P. niger* experiencing higher torsional loads on its femur than *P. concinna*, and that this would result in lower safety factors during walking. These data can provide insight into how variation within a given body design can contribute to changes in bone loading mechanics, and explores the possibility that pelvic girdle fusion, through its implications for limb bone loading, may have been a factor in constraining pleurodire turtles to primarily aquatic environments throughout their evolutionary history.

**MATERIALS AND METHODS**

*Experimental Animals*

Four West African black mud turtles (*P. niger*) (21.3 – 27.1 cm) were purchased from a commercial supplier (Turtles and Tortoises, Inc). Turtles were housed in pairs in 600-liter stock tanks equipped with pond filters, dry basking platforms, and a 200 W submerged heater (maintaining water at 25°C). Tanks were located in a temperature-controlled greenhouse facility that exposed turtles to ambient light patterns throughout the duration of experiments (November 2017 – February 2018). Turtles were fed commercial pellets (ReptoMin, Tetra, Blacksburg, VA, USA) *ad libitum*. All animal care and experimental
procedures were approved by the Institutional Animal Care and Use Committee at Clemson University (IACUC protocol 2017-062). Data from *P. niger* were compared with previously collected data on *P. concinna* (Butcher and Blob, 2008; Butcher et al., 2008).

*Surgical procedures*

All surgical procedures followed published methods (Biewener, 1992; Blob and Biewener, 1999), whereby strain gauges were attached surgically to the right femur of each animal using aseptic technique. To induce analgesia and a surgical plane of anesthesia, doses of 1 mg kg\(^{-1}\) butorphanol, 100 mg kg\(^{-1}\) ketamine and 1 mg kg\(^{-1}\) xylazine were injected into the proximal forelimb muscles, with supplemental doses provided as required.

Gauge attachment sites were exposed by making a medial incision along the ventral surface of the thigh at mid-shaft. Muscles along the ventral surface of the femur were retracted along the fascial plane between ambiens and the pubotibialis to expose the bone. Gauges were attached at mid-shaft by removing a ‘window’ of periosteum to expose the bone cortex. The femur was then gently scraped using a periosteal elevator and swabbed clean using a cotton-tipped applicator before the gauges were attached using a self-catalyzing cyanoacrylate adhesive (Duro\textsuperscript{TM} Superglue; Henkel Loctite Corp., Avon, OH, USA).
Gauges were attached to the femur in ‘posterior’ ‘ventral’ and ‘anterior’ positions (Romer, 1956), and were aligned (within 5°) with the long axis of the femur. We attached rosette (ROS) gauges (FRA-1-11) to the posterior and anterior positions, and the single element (SE) to the ventral position in all turtles but one, in which we replaced the anterior rosette with a single element gauge due to space constraints. After all gauges were in place, we passed lead wires from the gauges (336 FTE, etched Teflon; Measurements Group, Raleigh, NC, USA) subcutaneously through a small skin incision on the posterodorsal aspect of the medial thigh. Incisions were then sutured closed and lead wires were soldered into a microconnector and secured to the shell. Solder connections were strengthened with epoxy adhesive, and lead wires were protected by wrapping a self-adhesive bandage around the exposed portions, which was subsequently secured to the shell with tape. Turtles were allowed 1 – 2 days of recovery, after which in vivo strains were recorded over the course of one day.

In vivo strain data collection and analysis

Strain data were collected while turtles walked on a motorized treadmill. Most trials consisted of 5-9 footfalls of the right hindlimb during steady-speed walking (~0.1 ms⁻¹; 0.4-0.7 carapace lengths s⁻¹). Strain signals were conducted from gauges to Vishay conditioning bridge amplifiers (model 2120B; Measurements Group) via a shielded cable. Raw voltage signals were recorded at 2500 Hz after being sampled through an A/D converter (model PCI-6031 E; National Instruments Corp., Austin, TX, USA), calibrated
to microstrain ($\mu e = \text{strain} \times 10^{-6}$) and saved using custom LabVIEW™ routines (V. 6.1; National Instruments). Periods of rest and rehydration were given between trials, and temperatures were maintained by heat lamps at 25°C throughout the course of data collection.

High-speed (100Hz) lateral and dorsal view videos were collected in conjunction with strain recordings to document locomotor behavior and footfall patterns (Phantom V 5.1; Vision Research Inc., Wayne, NJ, USA). Video and strain data were synchronized using a trigger that simultaneously produced a 1.5 V pulse in strain recordings and a LED light in video recordings. We collected between 54 and 130 limb cycles per turtle, depending on their ability to maintain performance throughout data collection. Following completion of strain recordings, animals were euthanized by an overdose of a pentobarbital sodium solution (Beuthanasia®; 200 mg kg$^{-1}$ intraperitoneal injection) and placed in a freezer for later dissection and verification of gauge placement.

We followed standard conventions for the interpretation and analysis of strain data (Blob and Biewener, 1999; Butcher et al., 2008), whereby tensile strains are positive and compressive strains are negative. For our longitudinal (SE) strain data, the magnitudes of peak axial strains were determined for each gauge location for each cycle of the right hindlimb. The relative magnitude of tensile and compressive strains for each gauge location were then used to evaluate the loading regime of the bone throughout locomotion (pure bending vs a combination of bending and axial loads). For our ROS data, we followed published methods to calculate the magnitude and orientation of peak principal strains and shear strains (Biewener, 1992; Biewener and Dial, 1995; Carter,
These calculations allow for the evaluation of the importance of torsional loading in the femur. We defined the long axis of the femur as 0°, resulting in pure torsional loads showing principal strain orientations (\(\phi\)) of 45° when the femur is rotated clockwise and -45° when it has been rotated counterclockwise.

To evaluate the relationship between strain rate and magnitude in the femur of *P. niger*, we calculated longitudinal strain rate for 69 steps in three turtles (\(N = 23\) steps per turtle). We used the ‘ventral’ gauge location in analyses, as it consistently exhibited the highest ratio of tensile:compressive strains. Strain rate was calculated by determining the slope of a linear least-squares regression of strain magnitude on time during the loading portion of each footfall. We then regressed absolute strain magnitude on strain rate from the same steps to get the slope for the relationship between strain rate and magnitude.

Because strain gauges only record strain at the location to which the gauge has been applied, they do not necessarily record the maximum amount of strain that a bone experiences during loading. We used planar strain theory to estimate the cross-sectional strain distribution, the sites of maximum tensile and minimum compressive strains, and the location of the neutral axis of bending (Biewener, 1992; Carter et al., 1981; Lieberman et al., 2004; Verner et al., 2016). To apply this theory and estimate maximal loads, we dissected the muscles of the hindlimbs to measure physiological cross-sectional area (PCSA) and muscle leverage for each muscle (Table S1; Butcher and Blob, 2008; Mayerl et al., 2017), before removing and cleaning the femur and embedding it in fiberglass resin for planar strain analyses. Transverse sections were cut from each femur through the mid-shaft gauge locations, and the resulting cross-section was photographed.
using a digital camera with a macro lens. We analyzed these photographs in Adobe Photoshop to trace endosteal and periosteal outlines and mark the locations of the three gauges on the bone perimeter. The geometric data and longitudinal strain data for each femur were then input into analysis macros for the public domain software NIH Image for Macintosh (http://rsb.info.nih.gov/nih-image/) to calculate the location of the neutral axis of bending and the planar distribution of longitudinal strains through femoral cross sections (Lieberman et al., 2003; Lieberman et al., 2004).

We conducted planar strain analyses on a subset of steps to calculate peak values of tensile and compressive strain, and compared these to measured peak strains to determine the proportional increase in strain between recorded peaks and calculated peak magnitudes. We also evaluated shifts in the location and orientation of the neutral axis of the bone throughout a step by performing planar strain analyses at five time-points during a step (15%, 30%, 50%, 70% and 85% of stance) for six steps per individual (N = 24 steps total). We calculated safety factor for *P. niger* from the mean values of peak shear strain multiplied by a proportional value of strain increase determined from planar strain analyses (Blob and Biewener, 1999; Butcher et al., 2008), and used an estimate of the maximal load before failure from *P. concinna* (Butcher et al., 2008). Mean safety factor for *P. niger* was calculated as the mean safety factor for each individual and a ‘worst case’ safety factor was calculated using the highest value of shear strain following planar strain analyses.
Statistical analyses

We used linear mixed effects models (lme4; Bates et al., 2015) to test for differences between species in the coefficient of variation for individual turtles, as well as for differences in stance duration. We also used linear mixed effects models to test for a relationship between strain rate and strain magnitude in *P. niger*. Cohen’s *d* was used to calculate a measure of the effect size for any statistics we performed (effsize; R package version 0.7.0, https://CRAN.R-project.org/package=effsize). We also used a log linear model to examine the relationship between duty factor and stance duration (duty factor ~ log(stance)).

RESULTS

Locomotor strain patterns, magnitudes, and rate

Due to the limitations of placing strain gauges in living animals, not all surgical gauge placements were in the exact same location on the bone, so that caution should be exercised in making statistical comparisons between *P. niger* and *P. concinna* for specific gauge locations. However, the patterns of tensile and compressive strain at each recording location were consistent within individuals, enabling us to interpret the general pattern of femoral loading in both species.

Strain magnitudes and loading patterns were generally similar between *P. niger* and *P. concinna* (Table 1). For both species, strain traces typically showed only single peaks, similar to most other species (Biewener and Taylor, 1986; Blob and Biewener,
In *P. niger*, maximal principal, shear, and axial compressive strains occurred in synchrony at approximately 35-45% of stance, but tensile axial strains consistently reached their peak later, at approximately 45-65% of the cycle (Fig. 1). In contrast, strain peaks in *P. concinna* were generally synchronous for all loading regimes (Fig. 2). In general, both species of turtle exhibited φ angles (angle of principal tensile strain to the long axis of the bone) of approximately 45°, indicating that the femur was subject to substantial torsional loading. However, one individual *P. niger* exhibited φ angles of close to zero, with minimal amounts of shear strain (Table 2). We also observed variation in the loading regime of longitudinal strains in two of our turtles, which despite similar gauge placement along the posterior margin of the femur, exhibited primarily tensile, rather than compressive loading at this location (Table 1). The coefficient of variation (CV) in load magnitude within individuals was similar for both species (*P. niger* = 0.27 ± 0.05 SE; *P. concinna* = 0.33 ± 0.05 SE, p = 0.37, D = 0.34).

There was no relationship between maximum longitudinal strain magnitude and maximal strain rate in *P. niger* (All turtles: strain Magnitude ~ Strain rate + 1|Turtle + 1|Step; p = 0.19, Fig. 3), unlike the relationship found within *P. concinna* (Butcher et al., 2008). We found no substantial differences in mean stance duration between species (p = 0.28, D = 0.45, *P. niger* = 0.94 ± 0.16 sec; *P. concinna* = 1.20 ± 0.18 sec).

*Planar strain analyses and neutral axis orientation*
Planar strain analyses showed generally similar orientations of the neutral axis between the two species (Fig. 4). Both species generally showed a neutral axis orientation diagonally between the AP and DV axis, with the anteroventral aspect of the femur loaded in tension and the dorsal and posterior portions of the bone loaded in compression (Fig. 4). One turtle (Pn04) exhibited marked changes in NA orientation throughout stance, as the neutral axis was originally oriented diagonally between the AP and DV axis with the antero-dorsal aspect of the femur loaded in tension and the posterior and ventral portions oriented in compression. This orientation rotated to be similar to other turtles by mid-stance (Fig. 4). In both species, peak tensile strains occurred on the antero-ventral surface of the bone, with peak compressive strains occurring on the posterior and dorsal surfaces. Because of this, actual peak strains in the cooter femur were, on average, 1.43 times higher than recorded strains (Butcher et al., 2008). Calculated estimates of peak strains based on planar strain analyses were approximately 2.2 times higher than recorded strains.

Safety factors
To calculate safety factors, we used mechanical properties for the femur of *P. concinna* from Butcher et al. (2008, Table 2). Prior to calculating safety factors, peak strains were multiplied by 1.43 for *P. concinna* and 2.2 for *P. niger* to reflect results of planar strain analyses. Mean peak principal strain for both species was approximately 1200 µƐ, resulting in a safety factor of 7 for bending (Table 3). Although planar strain analyses in shear are not as accurate as they are for principal strains, they do provide a better estimate
than raw values (Verner et al., 2015), and we found that mean calculated shear strains were 2484.5 for *P. concinna* and 2259 for *P. niger*, resulting in similar safety factors of 3.8 and 4.1 respectively. The highest magnitude of calculated peak shear strain was 5315.6 μƐ in *P. concinna* and 5315.2 μƐ in *P. niger*, resulting in a worst-case safety factor estimate of 1.8 for both species.

**DISCUSSION**

Overall, we found that *in vivo* femoral bone strains were not substantially greater in the pleurodire *P. niger* than in the cryptodire *P. concinna*, despite our expectations for higher strains in pleurodires based on the fusion of their pelvic girdle to the shell. Similarly, both species exhibited generally similar bone loading regimes, including substantial bending and torsion. Both species of turtles generally exhibited ϕ angles of close to 45 degrees with greater shear strains than other vertebrates (Blob et al., 2014), and shear strains were more than double longitudinal strains and were also greater than peak principal strain magnitudes (Table 1). These loading patterns likely result from a combination of muscle action at the time of peak shear strain (near the beginning of stance) and the inwards rotational moment of the GRF acting on the femur (Butcher and Blob, 2008; Mayerl et al., 2017). We found that *P. niger* exhibited maximal tensile strains later in the limb cycle than maximal compressive strains, whereas *P. concinna* exhibited maximal longitudinal strains at approximately the same time (Fig. 2,3). This could be due to differences in
gauge placement between the taxa, or potentially to differences in muscle action as the limb shifts from stance to swing and the muscles on the dorsal and anterior surface of the femur contract, placing the ventral surface of the bone (where our gauges were located) in tension (Aiello et al., 2013). As pleurodires generally exhibit increased muscle use at this point in the limb cycle, this could explain why they show maximal tension then, rather than early in the cycle as with *P. concinna* (Mayerl et al., 2017). Both species of turtle also show patterns of longitudinal strain similar to other reptiles, whereby the ventral aspect of the femur experiences net tension (Table 1, Blob and Biewener, 1999; Blob and Biewener, 2001; Butcher and Blob, 2008; Sheffield et al., 2011). This could result from tension being maintained on the anteroventral surface of the bone due to the combined effects of femoral orientation and muscle contraction during stance, exceeding the moment imposed by gravity to load this surface in compression. A combination of *in vivo* strain, EMG, and XROMM recordings would be required to test this possibility.

These patterns in turtles are similar to the loading regime of alligators, and suggest that in addition to the substantial torsion, the femur is loaded in dorsoventral bending throughout stance (Blob and Biewener, 1999). This suggests that a combination of torsion and dorsoventral bending throughout stance is a common feature in sprawling species, and is not impacted by the fusion of the pelvis to the shell. After applying planar strain analyses, we also found that the two turtle species experience very similar safety factors in both bending (~7) and shear (~4). The femoral safety factors of both of these species lie within the range of other non-avian reptiles, and are generally higher than those found in birds and mammals (2-4, Alexander, 1981; Biewener, 1993; Biewener and
Dial, 1995; Blob and Biewener, 1999; Lanyon and Rubin, 1985). This could be due to differences in material properties of the bone, or differences in load magnitudes (Butcher et al., 2008; Blob et al., 2014).

The relationship between strain rate and strain magnitude

We also found no relationship between peak strain rate and peak strain magnitude (Fig. 3), which could be due to the substantially lower strain rates that *P. niger* experiences when compared with *P. concinna* or other tetrapods (Butcher et al., 2008; Aiello et al., 2015). Peak strain rates in *P. niger* never reached above 5,000 με sec\(^{-1}\), whereas in *P. concinna* strain rates approached 50,000 με sec\(^{-1}\) (Fig. 3). These differences were observed despite negligible differences in stance duration, suggesting that pleurodires have a lower strain rate despite taking steps of similar duration to those of the cryptodire (Fig. 4). *P. niger* may be planting the foot and loading their limb over a greater length of time, which could be tested by conducting force platform analyses (Butcher and Blob, 2008). Additionally, *P. niger* femora could have material properties that differ from those of *P. concinna* that would influence how strain magnitudes develop over a given step. Direct measurements of bone properties in *P. niger* could test this possibility.

Morphological and behavioral differences between *P. concinna* and *P. niger*

Bone loading patterns and magnitudes can be influenced by a wide range of factors, some of which might help to explain why *P. niger* does not exhibit higher torsional strains than *P. concinna* despite the fusion of the pelvis to the shell in pleurodires. For example,
curvature of the limb bones can reduce the variability of loads that bones experience (Moreno et al., 2008). Although the femur of *P. niger* is much more curved than the femur of *P. concinna* (C. Mayerl, personal observation), both species show similar coefficients of variation for their primary loads. This suggests that differences in bone shape between these two species do not differentially reduce variation in their loads, and may be instead have different functional consequences. However, as pleurodires use greater long axis rotation of the femur compared to cryptodires (Mayerl et al., 2016), the combination of greater curvature and rotation of femur could serve to reduce shear strains along the axis of the bone.

Bone strain during terrestrial locomotion is a product of both the moment that the GRF exerts on the bone as well as the moments exerted by limb muscles (Aiello et al., 2013; Alexander, 1974; Biewener, 1983; Biewener, 1989; Blob and Biewener, 1999). As such, differences in muscle use and posture can have profound impacts on bone strain, and might explain why we found no difference in femoral shear strains between *P. niger* and *P. concinna*. Previous work comparing muscle use during walking in pleurodire and cryptodire turtles has shown the pleurodires exhibit substantially different muscle use patterns than cryptodires (Mayerl et al., 2017). This is especially apparent in puboischiofemoralis internus, a muscle that serves only as a protractor in *P. concinna* but is active during stance as a hindlimb adductor in pleurodires and results in increased adduction of the femur (Mayerl et al., 2017). As a more upright posture has been associated with decreased levels of shear in mammals (Butcher et al., 2011; Copplö et
al., 2015), the increased depression of the femur in pleurodire turtles could facilitate reasonable shear strains during terrestrial locomotion.

*Individual variation in P. niger*

Although variation in bone loading regimes and magnitudes was similar between *P. niger* and *P. concinna*, we found substantial differences in bone loading patterns between individuals of *P. niger*. Although three *P. niger* individuals showed high levels of torsion, one turtle (*P. niger* 02) showed femoral loads that consisted almost entirely of bending, with high levels of longitudinal strain, a $\phi$ angle of close to zero, and very low shear strains. We also observed differences in patterns of longitudinal strain between our turtles, as two individuals experienced primarily tensile loads on the posterior aspect of the femur, and our other two individuals experienced primarily compressive loads at this location, despite apparently similar gauge placements (Table 1, Fig. 5). This high amount of interindividual variation with low levels of intraindividual variation suggests that individual *P. niger* turtles experience highly regular loading regimes, but that individuals can differ from each other and locomotor patterns may not be highly stereotyped for the species. Because selection often acts to decrease variance between individuals, and pleurodires have never radiated onto land (Bonin et al., 2006), it is possible that the high amount of interindividual variation we observed in terrestrial loading patterns in *P. niger* might be related to a lack of selection on terrestrial performance in this primarily aquatic lineage.
Conclusions

We found that femoral bone strains in *P. niger* were not substantially greater than those in *P. concinna*, contrary to expectations based on pelvic girdle fusion in *P. niger*. This suggests that pelvic girdle mobility might not contribute to decreasing twisting patterns of the femur, and that fusion of the pelvis to the shell might not have played a role in constraining pleurodires to primarily aquatic habitats throughout their evolutionary history. However, shear strain magnitudes in both species of turtle are much higher than those of other walking tetrapods, suggesting that the restriction of the vertebral column to the shell in turtles may indeed be a driving factor in why turtles experience such high shear strains when compared to other phylogenetic groups (Butcher et al., 2008; Blob et al., 2014). Alternatively, *P. niger* might compensate for the lack of mobility in its pelvis by adjusting behaviorally (differences in muscle use or posture), or through morphological features of the femur (more curved or different material properties) that better resist torsional loading. These possibilities could be tested through the integration of *in vivo* bone strain data with force plate measurements, X-Ray Reconstruction of Moving Morphology (XROMM) and electromyography (EMG) experiments to clarify the relative roles of posture, kinematics, and muscle use in determining the magnitudes and patterns of bone loading during locomotion (e.g., Aiello et al., 2013). We found that the loading regime of *P. niger* was broadly similar to other sprawling species, whereas *P. concinna* experiences a different loading regime, suggesting that the loading regime of this semiaquatic cryptodire may be the exception, rather than the rule regarding the loading regime of turtles during locomotion. A broader phylogenetic sample, or the use
of functional modelling, would also be useful in evaluating how representative these results are between cryptodire and pleurodire turtles.

ACKNOWLEDGEMENTS

I would like to thank A. Arellanez, D. Munteanu, A. Palecek A. Mckamy, N. Schneider, A. Sansone, and G. Forker for their assistance with surgery and collecting data.

FUNDING

This work was funded by Clemson University Creative Inquiry (Grant #479) to R. W. B.
REFERENCES


Blob, R. W. and Biewener, A. A. (1999). In vivo locomotor strain in the hindlimb bones of *Alligator mississippiensis* and Iguana iguana: implications for the evolution of


**Carter, D. R.** (1978). Anisotropic analysis of strain rosette information from cortical


Lieberman, D. E., Pearson, O. M., Polk, J. D., Demes, B. and Crompton, A. W.


Pace, C. M., Blob, R. W. and Westneat, M. W. (2001). Comparative kinematics of the
forelimb during swimming in red-eared slider (Trachemys scripta) and spiny softshell (Apalone spinifera) turtles. *J. Exp. Biol.* **204**, 3261–71.


Figure 1. Representative simultaneous strain recordings from three gauge locations on the femur of *P. niger* during three consecutive steps. A: principal strains from the posterior rosette gauge; B: axial strains from all three gauges; C: phi angle from the posterior rosette gauge; D: Shear strain from the posterior rosette gauge. Red lines
indicate primarily compressive loads, grey indicate intermediate loads, and black indicate primarily tensile loads. Dark grey shading indicates the stance phase for a single step at all gauge locations, with the light grey shading indicating swing phase for that step. Note that the y axis differs for each plot to facilitate presentation.
**Figure 2.** Representative strain recordings (simultaneous) from three gauge locations on the femur of *P. concinna* during three consecutive walking steps. Left: principal strains, angle of principal tensile strains from the femoral long axis (φt) and shear strains from ROS gauge recordings. Right: longitudinal strains from ‘dorsal’, ‘anterior’ and ‘ventral’ sites. Note that strain scales differ among panels to facilitate presentation. Shading follows conventions from Figure 1. εt and εc denote tensile and compressive (red line) principal strain traces, respectively.
Figure 3. The relationship between peak strain magnitude (in \( \mu \varepsilon \)) and peak strain rate (in \( \mu \varepsilon \, s^{-1} \)) for three \( P. \ niger \) turtles (N = 69 steps). All data are plotted as absolute values, and are colored by individual turtles. Solid lines indicate a linear least-squares regression for each turtle. There was no correlation between strain magnitude and strain rate for pooled data (p = 0.19, \( r^2 = -0.003 \)); or within a turtle (Pn01: p = 0.12, \( r^2 = 0.071 \); Pn02: p = 0.54, \( r^2 = -0.027 \); Pn03: p = 0.68, \( r^2 = -0.037 \)).
Figure 4. Graphical comparisons of cross-sectional planar strain analyses of femoral strain distributions calculated at five time increments (as a percentage of stance) during representative walking for one *P. concinna* (Pc05) and one *P. niger* (Pn04). The centroid of each cross-section is indicated by a black dot. Peak strains are indicated at either 30 or 50% of stance, depending on the individual. The neutral axis (NA) of bending is indicated by the red line that separates compressive (gray) from tensile (white) strains. Gauge locations are indicated by the black bars around the cortex of the femoral-
cross sections, with anatomical directions being labeled at the bottom of the image. Lines represent 100 µε increments.
Table 1. Peak axial ($\varepsilon_{\text{axial}}$), principal tensile ($\varepsilon_t$), principal compressive ($\varepsilon_c$) and shear strains from *P. niger* and *P. concinna* femur during walking.

<table>
<thead>
<tr>
<th>Gauge and Recordings</th>
<th>$P. niger$</th>
<th>$P. concinna$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal $\varepsilon_{\text{axial}}$ ($\mu\varepsilon$)</td>
<td>-486.2 ± 593.9 (255,5)</td>
<td></td>
</tr>
<tr>
<td>Anterior $\varepsilon_{\text{axial}}$ ($\mu\varepsilon$)</td>
<td>-177.3 ± 112.3 (404, 4)</td>
<td>218.9 ± 118.2 (242,5)</td>
</tr>
<tr>
<td>Ventral $\varepsilon_{\text{axial}}$ ($\mu\varepsilon$)</td>
<td>250.8 ± 242.3 (404, 4)</td>
<td>-104.5 ± 49.7 (263,6)</td>
</tr>
<tr>
<td>Posterior $\varepsilon_{\text{axial}}$ ($\mu\varepsilon$)</td>
<td>-324.5 ± 94.6 (157,2)</td>
<td>-</td>
</tr>
<tr>
<td>Posterior $\varepsilon_{\text{axial}}$ ($\mu\varepsilon$) 2</td>
<td>346.5 ± 120.5 (151, 2)</td>
<td></td>
</tr>
<tr>
<td>Dorsal $\varepsilon_t$ ($\mu\varepsilon$)</td>
<td>-</td>
<td>715.7 ± 11.5 (74, 2)</td>
</tr>
<tr>
<td>Dorsal $\varepsilon_c$ ($\mu\varepsilon$)</td>
<td>-</td>
<td>-825.9 ± 125.5 (74, 2)</td>
</tr>
<tr>
<td>Anterior $\varepsilon_t$ ($\mu\varepsilon$)</td>
<td>402.5 ± 107.6 (193, 2)</td>
<td>1310.2 ± 188.5 (81,1)</td>
</tr>
<tr>
<td>Anterior $\varepsilon_c$ ($\mu\varepsilon$)</td>
<td>-468.7 ± 212.3 (193, 2)</td>
<td>-1701.2 ± 212.1 (81, 1)</td>
</tr>
<tr>
<td>Ventral $\varepsilon_t$ ($\mu\varepsilon$)</td>
<td>-</td>
<td>833.4 ± 189.7 (76, 1)</td>
</tr>
<tr>
<td>Ventral $\varepsilon_c$ ($\mu\varepsilon$)</td>
<td>-</td>
<td>-975.7 ± 189.4 (76, 1)</td>
</tr>
<tr>
<td>Posterior $\varepsilon_t$ ($\mu\varepsilon$)</td>
<td>655.3 ± 212.1 (226, 2)</td>
<td>-</td>
</tr>
<tr>
<td>Posterior $\varepsilon_c$ ($\mu\varepsilon$)</td>
<td>-740.9 ± 148.1 (226, 2)</td>
<td>-</td>
</tr>
<tr>
<td>Dorsal $\phi$ (deg)</td>
<td>-</td>
<td>50.3 ± 8.9 (74, 2)</td>
</tr>
<tr>
<td>Anterior $\phi$ (deg)</td>
<td>48.4 ± 9.30 (193, 2)</td>
<td>42.6 ± 2.1 (81, 1)</td>
</tr>
<tr>
<td>Ventral $\phi$ (deg)</td>
<td>-</td>
<td>41.2 ± 2.7 (76, 1)</td>
</tr>
<tr>
<td>Posterior $\phi$ (deg)</td>
<td>43.19 ± 15.1 (226,2)</td>
<td>-</td>
</tr>
<tr>
<td>Dorsal Shear ($\mu\varepsilon$)</td>
<td>-</td>
<td>1441.3 ± 109.7 (74, 2)</td>
</tr>
<tr>
<td>Anterior Shear ($\mu\varepsilon$)</td>
<td>793.9 ± 251.9 (193, 2)</td>
<td>2934.9 ± 407.8 (81, 1)</td>
</tr>
<tr>
<td>Ventral Shear ($\mu\varepsilon$)</td>
<td>-</td>
<td>1788.1 ± 372.4 (76,1)</td>
</tr>
<tr>
<td>Posterior Shear($\mu\varepsilon$)</td>
<td>1225.8 ± 350.5 (226, 2)</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are means ($\pm$ s.d.) across all individuals for each gauge location. The number of steps analyzed and the number of individuals tested are indicated in parentheses. Positive angles for $\phi$ indicate inward (anterior) rotation for all gauge locations.
Table 2. Principal tensile (ε_t), principal compressive, (ε_c) and shear strains for an individual *P. niger* with irregular loading regime.

<table>
<thead>
<tr>
<th>Gauge and recording</th>
<th><em>P. niger</em> (Pn02)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior ɛ_t (με)</td>
<td>427.7 ± 83.7 (54)</td>
</tr>
<tr>
<td>Anterior ɛ_c (με)</td>
<td>-139.0 ± +64.5 (54)</td>
</tr>
<tr>
<td>Anterior φ (deg)</td>
<td>1.26 ± 5.9 (54)</td>
</tr>
<tr>
<td>Anterior Shear (με)</td>
<td>187.6 ± 101.4 (54)</td>
</tr>
</tbody>
</table>

Values are means (± s.d.) for Pn02. The number of steps analyzed are indicated in parentheses. Positive angles for φ indicate inward (anterior) rotation.
Table 3. Mechanical properties, estimated peak strains and mean safety factors for *P. niger* and *P. concinna*.

<table>
<thead>
<tr>
<th></th>
<th><em>P. niger</em></th>
<th><em>P. concinna</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bending yield strain (µε)</td>
<td>8316.0 ± 1176.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3)</td>
</tr>
<tr>
<td>Shear yield strain (µε)</td>
<td>9441.1 ± 1805.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3)</td>
</tr>
<tr>
<td>Proportional strain</td>
<td>2.24</td>
<td>1.43</td>
</tr>
<tr>
<td>increase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculated bending (µε)</td>
<td>1185.5</td>
<td>1209.2</td>
</tr>
<tr>
<td>Calculated shear (µε)</td>
<td>2259.0</td>
<td>2484.5</td>
</tr>
<tr>
<td>Femur bending mean SF</td>
<td>7.0</td>
<td>6.9</td>
</tr>
<tr>
<td>Femur shear mean SF</td>
<td>4.1</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Mechanical property values are mean ± s.d.; number of bones tested in parentheses.
APPENDICES
Appendix A

SUPPLEMENTARY MATERIAL – CHAPTER 2

Pelvic girdle mobility of cryptodire and pleurodire turtles during walking and swimming
Fig. S1. Pelvic girdle movements in the pleurodire turtle *Emydura subglobosa* while swimming (A) (*N* = 2 individuals, 18 cycles), and walking (B) (*N* = 2 individuals, 35 cycles). Solid lines represent mean traces for each motion, shading represents standard errors for each motion, and colors indicate different axes of rotation (black - roll; blue - pitch; red - yaw). Traces were normalized to the same duration. Vertical dashed lines represent the transition from stance to swing (walking) or from stroke to recovery (swimming). Very little rotation occurs during either behavior.
Movie 1. Animation of cryptodire turtle while walking and swimming, showing typical movements of the femur and pelvis during each behavior. Note the extensive pelvic girdle movements occurring during both behaviors.

[LINK FOR MOVIE 1]

Movie 2. Animation of pleurodire turtle while walking and swimming, showing typical movements of the femur and pelvis during each behavior. Note the lack of substantial pelvic girdle movements.

[LINK FOR MOVIE 2]

Movie 3. Animation of a cryptodire turtle while walking, highlighting the substantial ellipsoid movements of the pubis and ischium due to pelvic girdle rotation.

[LINK FOR MOVIE 3]
Appendix B

SUPPLEMENTARY MATERIAL: CHAPTER 3

*Hindlimb muscle function in turtles: is novel skeletal design correlated with novel muscle function?*
Figure S1. Illustrations of all hindlimb muscles in the cryptodire *T. scripta* (left) and the pleurodire *E. subglobosa* (right) which have shifted from the ancestral origin on the pelvis in cryptodires to an origin on the shell in pleurodires. A,B lateral view with femur protracted, anterior is on the left; C,D lateral view with femur retracted, anterior is on the left; E,F ventral view with femur protracted, anterior is to the top. Hatched areas in F indicate the attachment of the pelvis and muscles to the shell. Yellow, Puboischiofemoralis internus (PIFI); Red, Iliofemoralis (ILF); Blue, Femorotibialis (FT); Green, Flexor tibialis internus (FTI); Purple, Caudo-iliofemoralis (CIF). Pink muscles are those not examined in this study. A-D, Dorsal muscles, Iliotibialis (anterior), Iliofigularis (posterior). E,F, Ventral muscles, anterior to posterior, ambiens, pubo-tibialis, adductor femoris, ischiotrochantericus.
Figure S2. Isolated femorotibialis muscle (blue) in the cryptodire *T. scripta* (A) and pleurodire *E. globosa* (B). This muscle is not associated with the hip joint, and demonstrates no substantial change in muscle origin between the two lineages.
Table S1: Hindlimb muscle use data collected from cryptodire and pleurodire turtles while swimming and walking.

| Muscle | Swimming | | Walking | | | |
|---|---|---|---|---|---|
| | Cryptodire | Pleurodire | Cryptodire | Pleurodire |
| PIFI | 3, 44 | 3, 74 | 2, 32 | 3, 66 |
| ILF | 3, 48 | 3, 40 | 3, 47 | 1, 23 |
| FT | 3, 51 | 1, 28 | 3, 51 | 1, 26 |
| FTI | 3, 50 | 3, 69 | 3, 43 | 3, 78 |
| CIF | 2, 33 | 3, 65 | 2, 36 | 3, 43 |

Numbers for each lineage indicate sample sizes for each muscle during each behavior (individuals, cycles). PIFI, puboischiofemoralis internus; ILF, iliofemoralis; FT, femorotibialis; FTI, flexor tibialis internus; CIF, caudi-iliofemoralis.
Table S2. Muscle activity patterns during locomotion while swimming and walking in cryptodire and pleurodire turtles.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Action</th>
<th>Swim</th>
<th>Walk</th>
<th>Canon 1</th>
<th>Canon 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cryptodire</td>
<td>Pleurodire</td>
<td>Cryptodire</td>
<td>Pleurodire</td>
</tr>
<tr>
<td>PIFI</td>
<td>On</td>
<td>43.08 ± 1.38</td>
<td>48.35 ± 0.45</td>
<td>53.12 ± 1.22</td>
<td>77.96 ± 0.73</td>
</tr>
<tr>
<td></td>
<td>Off</td>
<td>82.80 ± 0.83</td>
<td>89.23 ± 0.27</td>
<td>86.53 ± 1.01</td>
<td>101.76 ± 0.32</td>
</tr>
<tr>
<td>ILF</td>
<td>On</td>
<td>35.37 ± 1.21</td>
<td>55.18 ± 1.76</td>
<td>46.83 ± 1.20</td>
<td>76.57 ± 0.99</td>
</tr>
<tr>
<td></td>
<td>Off</td>
<td>65.09 ± 1.07</td>
<td>84.11 ± 1.52</td>
<td>74.83 ± 1.27</td>
<td>98.35 ± 0.47</td>
</tr>
<tr>
<td>FT</td>
<td>On</td>
<td>5.24 ± 0.97</td>
<td>31.87 ± 1.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Off</td>
<td>38.54 ± 1.01</td>
<td>51.58 ± 1.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTI</td>
<td>On</td>
<td>-3.38 ± 0.74</td>
<td>-0.96 ± 0.66</td>
<td>-0.71 ± 0.86</td>
<td>0.49 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>Off</td>
<td>22.29 ± 1.21</td>
<td>18.43 ± 1.13</td>
<td>28.50 ± 0.93</td>
<td>53.60 ± 1.16</td>
</tr>
<tr>
<td>CIF</td>
<td>On</td>
<td>-4.23 ± 0.73</td>
<td>1.82 ± 0.50</td>
<td>-0.14 ± 0.39</td>
<td>1.50 ± 0.60</td>
</tr>
<tr>
<td></td>
<td>Off</td>
<td>19.94 ± 1.26</td>
<td>22.27 ± 0.61</td>
<td>30.73 ± 0.89</td>
<td>47.35 ± 1.48</td>
</tr>
</tbody>
</table>

Values are mean ± SE activity timing (in percentage of limb cycle, with 0 when the limb is fully protracted). Canonical values are loaded scores for each variable in the CDA.

PIFI, puboischiofemoralis internus; ILF, iliofemoralis; FT, femorotibialis; FTI, flexor tibialis internus; CIF, caudi-iliofemoralis.
Table S3. Kinematics of cryptodire and pleurodire turtles, with p values, overall effects size ($\Omega^2$), effects size of species (Cohen’s d) for each environment, as well as the canonical discriminant loadings for the overall data.

<table>
<thead>
<tr>
<th></th>
<th>Swimming</th>
<th></th>
<th></th>
<th></th>
<th>Walking</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cryptodire</td>
<td>Pleurodire</td>
<td>p</td>
<td>$\Omega^2$</td>
<td>D</td>
<td>Cryptodire</td>
<td>Pleurodire</td>
<td>p</td>
<td>$\Omega^2$</td>
<td>D</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Max Pro</td>
<td>63.4 ± 0.8</td>
<td>47.1 ± 0.5</td>
<td>&lt;0.001</td>
<td>0.861</td>
<td>2.333</td>
<td>69.6 ± 0.5</td>
<td>64.2 ± 0.7</td>
<td>0.094</td>
<td>0.842</td>
<td>0.867</td>
<td>0.83</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Max Ret</td>
<td>-11.4 ± 1.9</td>
<td>-26.6 ± 0.6</td>
<td>0.005</td>
<td>0.682</td>
<td>1.254</td>
<td>-1.4 ± 2.7</td>
<td>-13.2 ± 1.4</td>
<td>0.234</td>
<td>0.728</td>
<td>0.578</td>
<td>0.13</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Max El</td>
<td>-4.7 ± 0.6</td>
<td>-10.8 ± 0.6</td>
<td>0.029</td>
<td>0.710</td>
<td>0.949</td>
<td>-1.4 ± 0.4</td>
<td>-7.9 ± 0.4</td>
<td>&lt;0.001</td>
<td>0.685</td>
<td>1.646</td>
<td>0.05</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Max Dep</td>
<td>-21.3 ± 0.7</td>
<td>-28.1 ± 0.7</td>
<td>0.136</td>
<td>0.750</td>
<td>0.839</td>
<td>-18.8 ± 0.4</td>
<td>-26.0 ± 0.5</td>
<td>0.003</td>
<td>0.772</td>
<td>1.509</td>
<td>-0.18</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Knee Ext</td>
<td>109.4 ± 1.5</td>
<td>130.7 ± 1.0</td>
<td>0.001</td>
<td>0.805</td>
<td>-1.658</td>
<td>107.5 ± 2.3</td>
<td>121.6 ± 1.5</td>
<td>0.171</td>
<td>0.806</td>
<td>0.748</td>
<td>0.36</td>
<td>-0.33</td>
<td></td>
</tr>
<tr>
<td>Knee Flex</td>
<td>50.7 ± 1.0</td>
<td>64.8 ± 1.2</td>
<td>0.003</td>
<td>0.801</td>
<td>-1.177</td>
<td>49.7 ± 1.5</td>
<td>66.9 ± 1.6</td>
<td>0.068</td>
<td>0.888</td>
<td>-1.067</td>
<td>0.50</td>
<td>-0.67</td>
<td></td>
</tr>
<tr>
<td>Ankle Ext</td>
<td>81.6 ± 1.5</td>
<td>80.1 ± 1.2</td>
<td>0.382</td>
<td>0.688</td>
<td>0.100</td>
<td>93.5 ± 2.5</td>
<td>96.9 ± 2.1</td>
<td>0.877</td>
<td>0.695</td>
<td>-0.155</td>
<td>0.35</td>
<td>-0.58</td>
<td></td>
</tr>
<tr>
<td>Ankle Flex</td>
<td>30.6 ± 1.4</td>
<td>19.5 ± 1.2</td>
<td>0.402</td>
<td>0.879</td>
<td>0.781</td>
<td>34.1 ± 1.6</td>
<td>41.9 ± 1.9</td>
<td>0.428</td>
<td>0.708</td>
<td>-0.436</td>
<td>0.18</td>
<td>-0.26</td>
<td></td>
</tr>
<tr>
<td>Max Feath</td>
<td>67.3 ± 2.3</td>
<td>85.4 ± 1.2</td>
<td>0.004</td>
<td>0.757</td>
<td>-1.059</td>
<td>27.0 ± 1.3</td>
<td>25.2 ± 1.4</td>
<td>0.998</td>
<td>0.690</td>
<td>0.125</td>
<td>-1.18</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Min Feath</td>
<td>-0.4 ± 2.2</td>
<td>3.6 ± 1.3</td>
<td>0.343</td>
<td>0.622</td>
<td>-0.226</td>
<td>-4.9 ± 1.3</td>
<td>-9.7 ± 0.6</td>
<td>0.622</td>
<td>0.743</td>
<td>0.338</td>
<td>0.62</td>
<td>0.11</td>
<td></td>
</tr>
</tbody>
</table>
Values are mean angles ± SE. p values reported are those from mixed effects models, $\Omega^2$ provides an effects size for the overall model, and D represents the Cohen’s d value for the main effect in the model (species). N = 5 individuals, 84 cycles for cryptodire swimming, and 88 cycles for cryptodire walking; N = 6 individuals, 149 cycles for pleurodire swimming, and 116 cycles for pleurodire walking. Max Pro, Maximum protraction; Max Ret, maximum retraction; Max El, Maximum elevation; Max dep, Maximum depression; Knee Ext, Maximum knee extension; Knee Flex, Maximum knee flexion; Ankle Ext, maximum ankle extension; Ankle Flex, maximum ankle flexion; Max Feath, Maximum angle of pes; Min Feath, minimum angle of pes.
Appendix C

SUPPLEMENTARY MATERIAL: CHAPTER 4

Comparative stability and turning performance of swimming cryptodire and pleurodire turtles
Figure S1. Kinematic excursions of the left (solid lines) and right (dashed lines) limbs for *E. subglobosa* (green, N = 108 straight strokes, N = 45 turns), and *C. picta* (blue, N = 84 straight strokes, N = 43 turns). (A) Humeral angle during linear swimming, (B) Humeral angle during a turn; (C) Femoral angle during linear swimming; (D) Femoral angle during a turn. In C and D, the right limbs were the inner limbs during turns. A humeral or femoral angle of 0 degrees indicates a protracted limb parallel to the long axis of the body, with values greater than zero indicating greater retraction (and values less than zero for the forelimb indicating protraction such that the elbow is medial to the
shoulder). Shaded areas indicated ± 1 standard error. Vertical lines in (B) and (D) represent the mean end of the turn in *E. subglobosa* (green) and *C. picta* (blue). Note that because the maximum value for each trial does not always occur at the same percentage of the limb cycle, it is possible that calculated mean maximum values across all trials may be different than apparent maximum values seen in mean kinematic profiles.
Figure S2. Representative photos of *E. subglobosa* manus (A) and pes (C) compared with *C. picta* manus (B) and pes (D). The webbing in the manus of *E. subglobosa* is much more extensive than in *C. picta*. The blue outline in panel C indicates an example of how area was measured in ImageJ. Scale bar = 1 cm.
**Table S1.** Comparison of mean (± S.E.) values of stability and turning performance metrics for the turtles *E. subglobosa* (pleurodire) and *C. picta* (cryptodire) (*N*=4 individuals and ~ 100 linear cycles and 45 turns per species).

<table>
<thead>
<tr>
<th>Variable</th>
<th><em>E. subglobosa</em></th>
<th><em>C. picta</em></th>
<th>P</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heave Excursion (BL)</td>
<td>0.021 ± 0.001</td>
<td>0.033 ± 0.003</td>
<td>&lt;0.001</td>
<td>0.857</td>
</tr>
<tr>
<td>Pitch Excursion (deg)</td>
<td>4.83 ± 0.259</td>
<td>6.85 ± 0.499</td>
<td>0.194</td>
<td>0.761</td>
</tr>
<tr>
<td>Sideslip Excursion (BL)</td>
<td>0.019 ± 0.001</td>
<td>0.029 ± 0.002</td>
<td>&lt;0.001</td>
<td>0.799</td>
</tr>
<tr>
<td>Yaw Excursion (deg)</td>
<td>7.39 ± 0.319</td>
<td>12.32 ± 0.385</td>
<td>&lt;0.001</td>
<td>1.68</td>
</tr>
<tr>
<td>R (BL)</td>
<td>0.111 ± 0.012</td>
<td>0.251 ± 0.020</td>
<td>0.002</td>
<td>1.28</td>
</tr>
<tr>
<td>$R_{space}$</td>
<td>0.283 ± 0.044</td>
<td>0.436 ± 0.044</td>
<td>0.014</td>
<td>1.02</td>
</tr>
<tr>
<td>$\omega_{max}$ (deg/sec)</td>
<td>383.2 ± 9.83</td>
<td>310.5 ± 8.98</td>
<td>&lt;0.001</td>
<td>1.16</td>
</tr>
<tr>
<td>$\omega_{avg}$ (deg/sec)</td>
<td>229.8 ± 8.58</td>
<td>174.4 ± 8.70</td>
<td>&lt;0.001</td>
<td>1.29</td>
</tr>
</tbody>
</table>

R: Minimum radius of the turn

$\omega_{max}$: Maximum angular velocity of the turn

D: Cohen’s D, a measure of effect size
Table S2. Comparisons between turtle species of kinematic excursions (mean ± S.E. of maximum and minimum angles, in degrees) for all four limbs during stability trials, along with loadings of these variables for principal components analysis (figure 3).

<table>
<thead>
<tr>
<th></th>
<th>E. subglobosa</th>
<th>C. picta</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larm Max</td>
<td>91.02 ± 1.89</td>
<td>108.96 ± 5.22</td>
<td>0.446</td>
<td>-0.362</td>
<td>0.344</td>
</tr>
<tr>
<td>Larm Min</td>
<td>-6.87 ± 1.21</td>
<td>-25.15 ± 5.99</td>
<td>0.292</td>
<td>0.244</td>
<td>0.384</td>
</tr>
<tr>
<td>Rarm Max</td>
<td>94.50 ± 1.61</td>
<td>108.84 ± 5.50</td>
<td>0.521</td>
<td>0.242</td>
<td>-0.591</td>
</tr>
<tr>
<td>Rarm Min</td>
<td>-2.59 ± 0.99</td>
<td>-25.19 ± 6.91</td>
<td>0.16</td>
<td>0.549</td>
<td>-0.012</td>
</tr>
<tr>
<td>Lleg Max</td>
<td>132.62 ± 1.56</td>
<td>142.79 ± 1.17</td>
<td>0.406</td>
<td>-0.284</td>
<td>0.156</td>
</tr>
<tr>
<td>Lleg Min</td>
<td>57.16 ± 1.24</td>
<td>46.48 ± 1.38</td>
<td>0</td>
<td>0.287</td>
<td>0.344</td>
</tr>
<tr>
<td>Rleg Max</td>
<td>133.10 ± 1.32</td>
<td>142.45 ± 1.38</td>
<td>0.502</td>
<td>0.218</td>
<td>-0.298</td>
</tr>
<tr>
<td>Rleg Min</td>
<td>55.50 ± 1.10</td>
<td>44.42 ± 1.11</td>
<td>-0.033</td>
<td>0.489</td>
<td>0.391</td>
</tr>
</tbody>
</table>

Percent variation explained: PC1: 0.281; PC2: 0.266; PC3: 0.138

Larm: left arm; Rarm: right arm; Lleg: left leg; Rleg: right leg. Max: maximum retraction; Min: maximum protraction
**Table S3**: Limb velocity during linear swimming for *E. subglobosa* and *C. picta*, in deg sec\(^{-1}\)

<table>
<thead>
<tr>
<th>Limb</th>
<th><em>E. subglobosa</em></th>
<th><em>C. picta</em></th>
<th>P</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forelimb</td>
<td>368.75 ± 36.84</td>
<td>401.09 ± 35.43</td>
<td>0.08</td>
<td>0.003</td>
</tr>
<tr>
<td>Hindlimb</td>
<td>306.04 ± 21.63</td>
<td>291.86 ± 23.44</td>
<td>0.39</td>
<td>0.025</td>
</tr>
</tbody>
</table>

D: Cohen’s D, a measure of effect size
Table S4. Comparisons between turtle species of kinematic excursions (mean ± S.E. of maximum and minimum angles, in degrees) for all four limbs during turning performance trials, along with loadings of these variables for principal components analysis (figure 3).

<table>
<thead>
<tr>
<th></th>
<th>E. subglobosa</th>
<th>C. picta</th>
<th>LD1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larm Max</td>
<td>123.67 ± 1.72</td>
<td>124.53 ± 2.96</td>
<td>0.05</td>
</tr>
<tr>
<td>Larm Min</td>
<td>-29.89 ± 1.25</td>
<td>-36.17 ± 1.85</td>
<td>-0.52</td>
</tr>
<tr>
<td>Rarm Max</td>
<td>74.97 ± 4.15</td>
<td>68.45 ± 4.61</td>
<td>-0.20</td>
</tr>
<tr>
<td>Rarm Min</td>
<td>11.18 ± 2.35</td>
<td>7.01 ± 2.76</td>
<td>-0.22</td>
</tr>
<tr>
<td>Lleg Max</td>
<td>139.16 ± 2.89</td>
<td>136.64 ± 3.93</td>
<td>-0.10</td>
</tr>
<tr>
<td>Lleg Min</td>
<td>26.97 ± 1.46</td>
<td>31.33 ± 1.28</td>
<td>0.42</td>
</tr>
<tr>
<td>Rleg Max</td>
<td>109.24 ± 3.41</td>
<td>121.84 ± 4.01</td>
<td>0.45</td>
</tr>
<tr>
<td>Rleg Min</td>
<td>28.46 ± 1.23</td>
<td>30.35 ± 1.01</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Larm: left arm; Rarm: right arm; Lleg: left leg; Rleg: right leg. Max: maximum retraction; Min: maximum protraction

The left limbs are the outer limbs for all turns.
**Table S5**: Limb velocity during turns by *E. subglobosa* and *C. picta*, in deg sec$^{-1}$

<table>
<thead>
<tr>
<th>Limb</th>
<th><em>E. subglobosa</em></th>
<th><em>C. picta</em></th>
<th>P</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left (outer) forelimb</td>
<td>504.15 ± 39.19</td>
<td>418.36 ± 39.34</td>
<td>0.12</td>
<td>0.79</td>
</tr>
<tr>
<td>Right (inner) forelimb</td>
<td>203.65 ± 19.26</td>
<td>157.22 ± 19.51</td>
<td>0.09</td>
<td>0.48</td>
</tr>
<tr>
<td>Left (outer) hindlimb</td>
<td>368.74 ± 15.20</td>
<td>270.31 ± 15.5</td>
<td>&lt;0.001</td>
<td>1.06</td>
</tr>
<tr>
<td>Right (inner) hindlimb</td>
<td>267.27 ± 24.55</td>
<td>239.38 ± 24.71</td>
<td>0.42</td>
<td>0.34</td>
</tr>
</tbody>
</table>

D: Cohen’s D, a measure of effect size
Appendix D

SUPPLEMENTARY MATERIAL: CHAPTER 5

Bone loading mechanics during locomotion in pleurodire turtles
Table S1. Muscle property data.

<table>
<thead>
<tr>
<th></th>
<th>PIFI</th>
<th>ILF</th>
<th>FT</th>
<th>FTI-D</th>
<th>FTI-V</th>
<th>CIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCSA</td>
<td>0.79 ± 0.06</td>
<td>0.06 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Pro/Ret</td>
<td>0.15 ± 0.04</td>
<td>0.15 ± 0.02</td>
<td>-</td>
<td>0.12 ± 0.05</td>
<td>0.09 ± 0.02</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>Ab/Add</td>
<td>0.14 ± 0.03</td>
<td>0.19 ± 0.04</td>
<td>-</td>
<td>0.16 ± 0.02</td>
<td>0.16 ± 0.03</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>Rotation</td>
<td>0.10 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flex/Ext</td>
<td>-</td>
<td>-</td>
<td>0.12 ± 0.03</td>
<td>0.21 ± 0.03</td>
<td>*</td>
<td>-</td>
</tr>
</tbody>
</table>

Values reported are means ± d.d. (PCSA in m² normalized to femur length, Mechanical advantage is dimensionless, N = 4 P. niger). PIFI, puboishiofemoralis internus; ILF, iliofemoralis; FT, femorotibialis; FTI, flexor tibialis internu; CIF, caudi-iliofemoralis. PCSA, physiological cross sectional area; Pro/Ret, MA for femoral protraction or retraction; Ab/Add, MA for femoral abduction or adduction; Rotation, MA for femoral long axis rotation; Flex/Ext, MA for knee flexion or extension. *, MA for FTI-D and FTI-V is the same for Flex/Ext as they insert on a tendon proximal to the knee.