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COMPARATIVE FORELIMB MUSCLE FUNCTION IN TURTLES: TESTS OF ENVIRONMENTAL VARIATION AND NEUROMOTOR CONSERVATION

A Dissertation
Presented to
the Graduate School of
Clemson University

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

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by
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May 2011

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ABSTRACT

Novel locomotor functions in animals may evolve through changes in morphology, muscle activity, or a combination of both. The idea that new functions or behaviors can arise solely through changes in structure, without concurrent changes in the patterns of muscle activity that control movement of those structures, has been formalized as the ‘neuromotor conservation hypothesis’. In vertebrate locomotor systems, evidence for neuromotor conservation is found across transitions in terrestrial species and into fliers, but transitions in aquatic species have received little comparable study to determine if changes in morphology and muscle function were coordinated through the evolution of new locomotor behaviors. Understanding how animals move has long been an important component of integrative comparative biology and biomechanics. This topic can be divided into two components, the motion of the limbs, and the muscles that move them. Variation in these two parameters of movement is typically examined at three levels, intraspecfic studies of different behaviors, and interspecific studies on either the same or different behaviors.

My dissertation is a compilation of four studies that examined forelimb kinematics and motor control across locomotor modes in freshwater and marine turtles to determine how muscle function is modulated in the evolution of new locomotor styles. First, I described patterns of forelimb motion and associated patterns of muscle activation during swimming and walking in a generalized freshwater turtle species (*Trachemys scripta*) to show how muscle function is modulated to accommodate the different performance demands imposed by water and land. Second, I examined whether differences in muscle function are correlated with changes in limb morphology and locomotor style by comparing forelimb kinematics and motor patterns of swimming from rowing *Trachemys scripta* to those of flapping sea turtles (*Caretta caretta*). Next, I quantified forelimb kinematics of swimming in the freshwater turtle species *Carettochelys insculpta*, describing how it uses synchronous forelimb movements to swim and whether these motions are actually similar to the flapping kinematics of sea turtles (*Caretta caretta*) or if they more closely resemble the kinematics of freshwater species with which they are more phylogenetically similar. I also compared the kinematics of rowing in *Trachemys scripta* and the highly aquatic Florida softshell turtle (*Apalone ferox*). Finally, I compared patterns of forelimb muscle activation for four species of turtles to determine whether the chelonian lineage shows evidence of neuromotor conservation across the evolution of different locomotor modes. Data from these studies help improve our understanding of how new forms of quadrupedal locomotion have evolved.

DEDICATION

This dissertation is dedicated to my loving husband and best friend Gabriel and my little loves, Bosco, Russell, and Emyli. Thanks for always supporting me, encouraging me, making me smile and laugh in good times and bad, and most of all, thanks for loving me.

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I am particularly grateful to Dr. P. Pritchard (Chelonian Research Institute) for allowing me to dissect a preserved *Carettochelys* specimen when no one else would. Research was supported by SICB and Sigma Xi Grants-in-Aid-of-Research. Travel awards from SICB, SSAR, ASIH, and the Department of Biological Sciences enabled me to present my work at scientific conferences.

It is sometimes hard for those outside of academia to fully appreciate the distinctive rewards and challenges inherent to this career choice. This is especially true, I feel, during the grueling years we spend working on a dissertation. For their support and understanding over the past seven years I am incredibly grateful to the Biological Sciences graduate students. In particular, I thank Dr. S. J. Hankison and S. M. Kawano. I also extend special thanks to my grandparents, R. D. and C. M. Vogel, for their encouragement and support.

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CHAPTER ONE

INTRODUCTION

A major focus of evolutionary studies of musculoskeletal function is understanding how changes in anatomical structures are correlated with changes in muscle activity patterns during evolutionary changes in function or behavior. Novel behaviors can arise through modification of structures, modification of patterns of muscle activation, or some combination of both (Biewener and Gillis, 1999; Gillis and Blob, 2001; Blob et al., 2008). Despite dramatic variations in structure and function across vertebrate taxa, remarkably similar patterns of muscle activation have been documented across taxa that span diverse ranges of behavior in both feeding and locomotor systems (Peters and Goslow, 1983; Goslow et al., 1989; Westneat and Wainwright, 1989; Dial et al., 1991; Fish, 1996; Goslow et al., 2000). Such studies led to the hypothesis that patterns of neuromotor control often are conserved evolutionarily across behavioral transitions, even when morphological changes are dramatic (e.g., legs to wings: Jenkins and Goslow, 1983; Dial et al., 1991); this hypothesis is known as the ‘neuromotor conservation hypothesis’ (see Smith, 1994 for review). Although a number of its invocations have been criticized (Smith, 1994), this hypothesis has inspired numerous studies seeking to explain and understand the evolutionary diversity of functional performance (Jenkins and Goslow, 1983; Peters and Goslow, 1983; Goslow et al., 1989; Dial et al., 1991; Reilly and Lauder, 1992; Lauder and Reilly, 1996; Goslow et al., 2000). Initial studies of neuromotor conservation in tetrapod locomotion focused on terrestrial limb use and on transitions to flight (Jenkins and Weijs, 1979; Jenkins and Goslow, 1983; Peters and Goslow, 1983; Goslow et al., 1989; Dial et al., 1991; Goslow et al., 2000). However, dramatic structural changes also can be found through the evolution of locomotion in lineages of aquatic tetrapods (Fish, 1996), and whether neuromotor activation patterns were conserved through such transitions is unknown.

Vertebrate limbs have diversified considerably through the course of evolution, yielding a wide range of forms including the legs of terrestrial taxa, the flippers of aquatic taxa, and the wings of aerial taxa. While some of these structures are specialized for use in specific habitats, others are used by species across multiple habitats. Animals move through their environment to perform a wide range of crucial tasks, ranging from acquiring food, to finding mates, to avoiding and escaping predators. The physical characteristics of locomotor environments strongly influence the functional demands that the musculoskeletal systems of animals must satisfy (Gillis, 1998; Gillis and Biewener, 2000; Gillis and Blob, 2001; Higham and Jayne, 2004; Blob et al., 2008; Pace and Gibb, 2009). While species that live in a restricted range of habitats may show specializations that facilitate locomotor performance under specific physical conditions, species that live in or traverse multiple habitats typically use a single set of locomotor structures to meet potentially disparate functional requirements

(Gillis and Biewener, 2002; Daley and Biewener, 2003; Biewener and Daley, 2007).

In particular, semi-aquatic species that regularly move both through water and over land occur in every major group of vertebrates (i.e., fishes, amphibians, mammals, non-avian reptiles, and birds). Given the differences in viscosity, density, and the effects of gravity between these habitats, the functional demands placed on the musculoskeletal system are expected to be very different between aquatic and terrestrial locomotion (Dejours et al., 1987; Denny, 1993; Vogel, 1994; Gillis and Blob, 2001; Alexander, 2003; Horner and Jayne, 2008). However, little is known about how animals adjust musculoskeletal function to meet the differing demands of water and land.

Movement through aquatic habitats, in particular, is of considerable interest because animals that swim using appendages (e.g., fins or limbs), do so by way of rowing and/or flapping motions. While the specific motions may vary in different species, the presence in many taxa of generally similar limb motions provides an opportunity to study neuromotor conservation. Rowing is characterized by anteroposterior oscillatory motions of the limbs with distinct recovery and power strokes (Blake, 1979; Blake, 1980; Vogel, 1994; Walker and Westneat, 2000), whereas flapping is characterized by dorsoventral oscillatory motions of the limbs, in which a distinct recovery stroke may not be present (Aldridge, 1987; Rayner, 1993; Walker and Westneat, 1997; Wyneken, 1997; Walker and Westneat, 2000). Aquatic locomotion via rowing and flapping has been reported for a diverse range of taxa, including invertebrates (Plotnick, 1985; Seibel et al., 1998), fishes (Walker and Westneat, 2000; Walker, 2002; Walker and Westneat, 2002a; Walker and Westneat, 2002b), turtles (Davenport et al., 1984; Pace et al., 2001), birds (Baudinette and Gill, 1985), and mammals (Feldkamp, 1987; Fish, 1993; Fish, 1996).

Rowing and flapping fishes, in particular, have provided a productive system in which to examine the functional consequences and correlates of these two methods of swimming. Flapping has been shown to be a more energetically efficient mode of swimming than rowing, regardless of swimming speed (Walker and Westneat, 2000). This suggests that flapping should be employed by species that require energy conservation (Walker and Westneat, 2000), such as those that swim great distances. However, rowing appendages were found to generate more thrust during the power stroke, and to be better for maneuvers such as accelerating, braking, and turning (Walker and Westneat, 2000), suggesting that species that live in aquatic environments that require substantial maneuvering should employ rowing. A strong correlation between swimming mode and limb morphology also exists, with rowing appendages typically distally expanded or paddle shaped and flapping appendages typically distally tapering and wing-shaped (Walker, 2002; Walker and Westneat, 2002a; Walker and Westneat, 2002b). Another pattern associated with this dichotomy in swimming modes is that many rowing species are semi-aquatic. Semi-aquatic animals must function effectively on land, as well as in water, and limbs suited for rowing are better suited for terrestrial locomotion than those used for flapping (Vogel, 1994; Fish, 1996; Walker and Westneat, 2000). Moreover, animals for which forelimbs have evolved into specialized foreflippers used in aquatic flapping are rarely adept at terrestrial locomotion (e.g., pinnipeds) (Feldkamp, 1987; Renous and Bels, 1993; Fish, 1996).

Understanding how animals move has long been an important component of integrative comparative biology and biomechanics. This topic can be divided into two components, the motion of the limbs, and the muscles that move them. Variation in these two parameters of movement is typically examined at three levels, intraspecific studies of different behaviors, interspecific studies on similar behaviors, or interspecific studies on different behaviors.

Turtles are an excellent group in which to examine questions about musculoskeletal function because they provide several advantages with regard to environmentally correlated modulation of motor patterns and neuromotor conservation in the evolution of new locomotor behaviors. First, many species of turtles regularly perform both aquatic and terrestrial locomotion as part of their natural behaviors, with many species spending substantial amounts of time in both types of environments (Cagle, 1944; Bennett et al., 1970; Gibbons, 1970; Zug, 1971; Davenport et al., 1984; Ernst et al., 1994; Gillis and Blob, 2001; Blob et al., 2008). Additionally, because all turtles have a rigid shell comprised of fused vertebrae, ribs and dermal elements, movement of the body axis is precluded (Zug, 1971; Wyneken, 1997; Pace et al., 2001; Blob et al., 2008).

Thus, turtles represent an ideal group in which to study appendage-based locomotion because propulsive forces are generated exclusively by the limbs in any habitat (Zug, 1971; Wyneken, 1997; Pace et al., 2001; Blob et al., 2008; Rivera et al., 2011) Consequently, evaluations of differences in limb motor patterns across taxa should not be confounded significantly by the contributions of other structures to propulsion, like flexible bodies, tails, or specialized fins (Blake et al., 1995; Walker, 2000; Fish, 2002; Fish and Nicastro, 2003; Rivera et al., 2006).

In addition, turtles display a diverse range of locomotor styles and associated limb morphology. While there are many differences among aquatic turtle species (>200 species) with regard to their locomotion in aquatic habitats (Webb, 1962; Zug, 1971; Walker, 1973; Davenport et al., 1984; Pace et al., 2001; Blob et al., 2008; Renous et al., 2008), one of the most striking examples is in the use of rowing versus flapping in swimming taxa. Asynchronous rowing is the more common and ancestral form of swimming in turtles (Joyce and Gauthier, 2004), and has been reported to be used exclusively by all but one freshwater species (Fig. 1.1). In rowing turtles, the forelimb of one side moves essentially in phase with the contralateral hindlimb, so that forelimbs (and hindlimbs) of diagonally opposite limbs move asynchronously (Pace et al., 2001; Rivera et al., 2006; Rivera et al., 2011). Rowing species also tend to possess moderate to extensive webbing between the digits of the forelimb and hindlimb (Pace et al., 2001) [i.e., distally expanded and paddle-shaped; (Walker and Westneat,

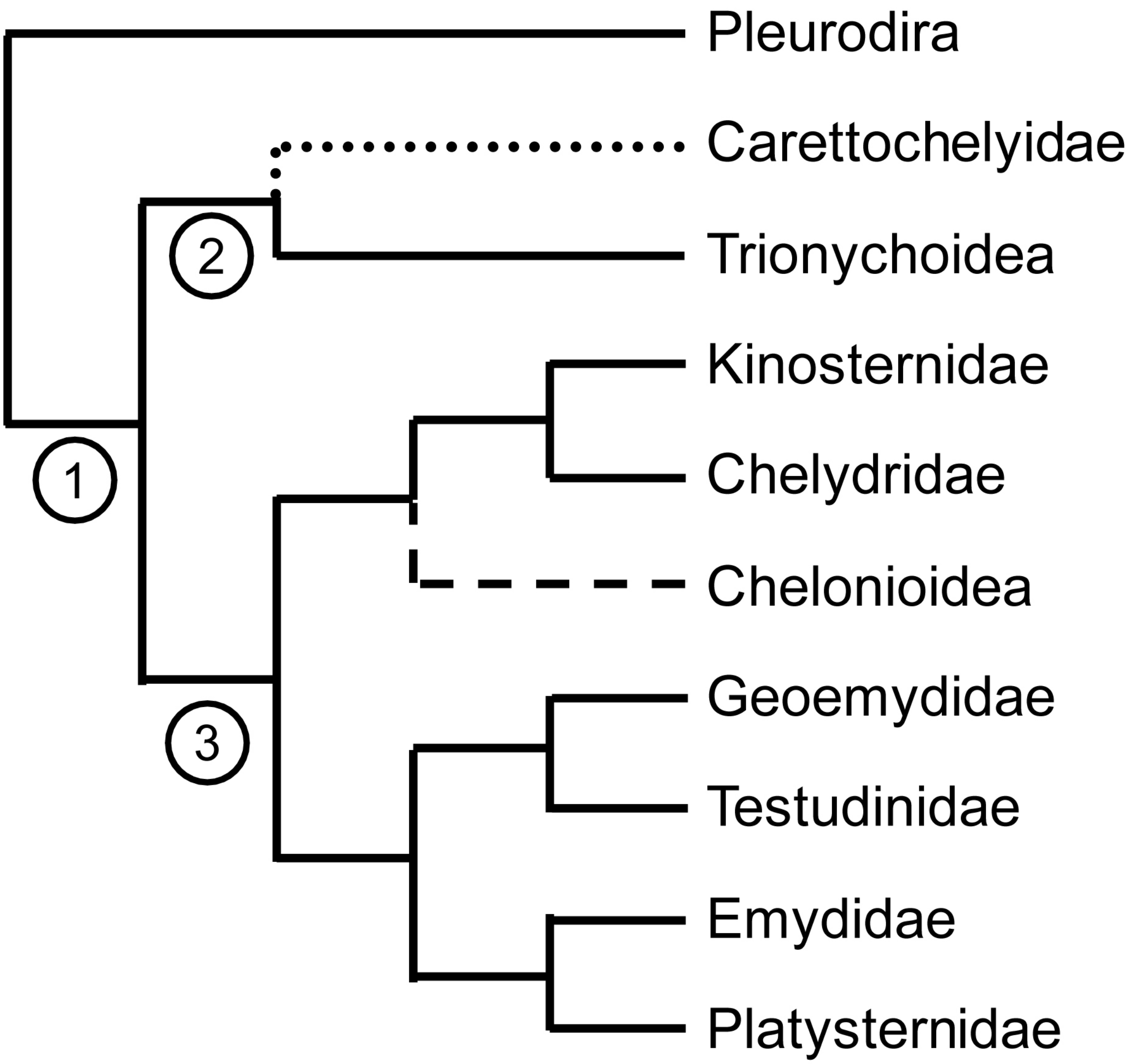

Fig. 1.1. Recent phylogeny of turtles, based on fourteen nuclear genes, showing familial relationships. Solid lines indicate asynchronous anteroposterior rowing motions of forelimbs and hindlimbs for swimming (presumptive ancestral condition), dashed line indicates synchronous dorsoventral flapping motions of forelimbs for swimming in sea turtles (derived), and dotted line indicates swimming in *Carettochelys insculpta* (the only extant member of the family Carettochelyidae, and only freshwater turtle species with forelimbs modified into flippers that swims using synchronous forelimb motions). The family Emydidae includes *Trachemys scripta*, Chelonioidea includes *Caretta caretta*, and Trionychoidea includes *Apalone ferox*. Branch lengths do not reflect time since divergence. Time since divergence of focal lineages is indicated at nodes: 1 = 175 mya; 2 = 155 mya; 3 = 94 mya. Phylogeny based on Barley et al. (Barley et al., 2010). Estimates of divergence times based on Near et al. (Near et al., 2005).

2002a)]. Synchronous flapping is a much rarer locomotor style used by turtles, definitively employed by the seven extant species of sea turtle (Wyneken, 1997; Fig. 1.1). Flapping turtles swim via synchronous motions of forelimbs that have been modified into flat, elongate, semi-rigid flippers [i.e., distally tapering wing-like appendages; (Walker and Westneat, 2002a)]. Foreflippers may produce thrust on both upstroke and downstroke, and while hindlimbs can aid in propulsion, flapping species commonly use swimming modes (i.e., aquatic flight) in which hindlimbs have a negligible propulsive role (Walker, 1971; Walker, 1973; Davenport et al., 1984; Renous and Bels, 1993; Walker and Westneat, 2000). In addition, synchronous flapping-style swimming has also been reported for a single freshwater species, the pig-nosed turtle *Carettochelys insculpta* (Walther, 1921; Rayner, 1985; Georges et al., 2000; Walker, 2002), which would represent an independent convergence on this swimming style within the chelonian lineage (Fig. 1.1). *Carettochelys insculpta* is the sole extant member of the carettochelyid lineage that forms the sister taxon to the trionychid clade (Engstrom et al., 2004; Fujita et al., 2004; Iverson et al., 2007; Fig. 1.1). While trionychids are highly specialized rowers with extensive webbing between the digits of the forelimb (Pace et al., 2001), this morphology appears even further hypertrophied in *C. insculpta* through elongation of both the digits and webbing, so that the forelimbs of this species converge on at least a superficial resemblance to the foreflipper anatomy of sea turtles (Walther, 1921). Yet, while described as using flapping forelimb motions (Rayner, 1985; Ernst and Barbour, 1989; Georges et al., 2000), kinematic measurements from *C. insculpta* are not currently available that would allow quantitative comparisons with flapping by sea turtles and evaluations of the similarity of these purportedly convergent locomotor styles.

Despite the dramatic differences in external morphology and humerus shape between the forelimbs of rowing and flapping turtles, all turtles share the same basic limb musculature [i.e., no major muscles were lost or added in the evolution of aquatic flight (Walker, 1973)]. This means that rowers and flappers with disparate limb morphology must execute their different styles of swimming either strictly as a mechanical consequence of those morphological differences (i.e., without changes in the underlying motor patterns), or through a combination of differences in morphology as well as motor patterns. The latter would indicate a lack of conservation, while the former would provide support for the hypothesis of neuromotor conservation in the evolution of flapping. While evidence for neuromotor conservation is found across terrestrial and aerial locomotor modes (Jenkins and Goslow, 1983; Dial et al., 1991; Goslow et al., 2000), few studies have examined this for swimming, particularly between aquatic rowing and flapping. The extent to which divergent motor patterns contribute to the diversity in locomotor behavior used by swimming turtles has not been evaluated (Blob et al., 2008). Comparisons of forelimb motor patterns across taxa that swim via rowing versus flapping would, therefore, allow evaluations of how divergence in limb neuromotor control contributes to divergence of limb kinematics and locomotor behavior through evolution in this lineage.

In my dissertation research, I conducted a series of studies that examined forelimb kinematics and motor control across habitats in a single turtle species and across multiple swimming styles in four species of turtles. The primary goal of this comparative approach was to investigate how different swimming styles have evolved among turtles and whether there is evidence supporting the hypothesis of neuromotor conservation within this distinctive lineage of tetrapods. Chapter 2 addresses intraspecific variation in kinematics and motor patterns, whereas Chapters 3, 4, and 5 address interspecific variation. Chapter 2 examines how muscle function is modulated to accommodate different performance demands by comparing the motor patterns of forelimb muscles in a generalized freshwater turtle, *Trachemys scripta* (red-eared slider turtle), during aquatic and terrestrial locomotion. Chapter 3 investigates whether differences in muscle function are correlated with changes in limb morphology and locomotor behavior by comparing forelimb kinematics and motor patterns of swimming from a generalized rower (*Trachemys scripta*) to those of flapping loggerhead sea turtles (*Caretta caretta*). Chapter 4 presents the first quantification of swimming kinematics in the pig-nosed turtle (*Carettochleys insculpta*) and describes how it uses synchronous forelimb movements to swim and whether these motions are actually similar to the flapping kinematics of sea turtles (*Caretta caretta*) or if they more closely resemble the kinematics of species with which they are more phylogenetically similar (Fig. 1.1). Chapter 4 also presents a comparison of rowing between *Trachemys scripta* and the highly aquatic Florida softshell turtle (*Apalone ferox*). Chapter 5 is the final component of my dissertation, in which I compare patterns of forelimb muscle activation for four species of turtle to determine whether this lineage shows evidence of neuromotor conservation across the evolution of different locomotor modes, including comparisons of drastically different rowing versus flapping, as well as more subtle comparisons of different forms of rowing.

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CHAPTER TWO

FORELIMB KINEMATICS AND MOTOR PATTERNS OF THE SLIDER TURTLE (*TRACHEMYS SCRIPTA*) DURING SWIMMING AND WALKING: SHARED AND NOVEL STRATEGIES FOR MEETING LOCOMOTOR DEMANDS OF WATER AND LAND

Abstract

Turtles use their limbs during both aquatic and terrestrial locomotion, but water and land impose dramatically different physical requirements. How must musculoskeletal function be adjusted to produce locomotion through such physically disparate habitats? I address this question by quantifying forelimb kinematics and muscle activity during aquatic and terrestrial locomotion in a generalized freshwater turtle, the red-eared slider (*Trachemys scripta*), using digital high-speed video and electromyography (EMG). Comparisons of my forelimb data to previously collected data from the slider hindlimb allow me to test whether limb muscles with similar functional roles show qualitatively similar modulations of activity across habitats. The different functional demands of water and air lead to a prediction that muscle activity for limb protractors (e.g., latissimus dorsi and deltoid for the forelimb) should be greater during swimming than during walking, and activity in retractors (e.g., coracobrachialis and pectoralis for the forelimb) should be greater during walking than during swimming. Differences between aquatic and terrestrial forelimb movements are reflected in temporal modulation of muscle activity bursts between environments, and in some cases the number of EMG bursts as well. While patterns of

modulation between water and land are similar between the fore- and hindlimb in *T. scripta* for propulsive phase muscles (retractors), I did not find support for the predicted pattern of intensity modulation, suggesting that the functional demands of the locomotor medium alone do not dictate differences in intensity of muscle activity across habitats.

Introduction

Animals move through their environment to perform a wide range of crucial tasks, ranging from acquiring food, to finding mates, to avoiding and escaping predators. The physical characteristics of locomotor environments strongly influence the functional demands that the musculoskeletal systems of animals must satisfy (Gillis, 1998a; Gillis and Biewener, 2000; Gillis and Blob, 2001; Higham and Jayne, 2004; Blob et al., 2008; Pace and Gibb, 2009). While species that live in a restricted range of habitats may show specializations that facilitate locomotor performance under specific physical conditions, species that live in or traverse multiple habitats must use a single set of locomotor structures to meet potentially disparate functional requirements (Gillis and Biewener, 2002; Daley and Biewener, 2003; Biewener and Daley, 2007).

One of the most common ways in which animals encounter locomotor environments with divergent demands is through the use of both aquatic and terrestrial habitats. Species that regularly move both through water and over land occur in every major group of vertebrates (i.e., fishes, amphibians, mammals, non-avian reptiles, and birds). Given the differences in viscosity, density, and the effects of gravity between these habitats, the functional demands placed on the musculoskeletal system are expected to be very different between aquatic and terrestrial locomotion (Horner and Jayne, 2008). How do animals adjust musculoskeletal function to meet the differing demands of water and land?

Previous studies have highlighted three general neuromuscular strategies for accommodating divergent demands (Biewener and Gillis, 1999; Gillis and Blob, 2001; Blob et al., 2008). First, there might be no change in muscle activation patterns between behaviors. This pattern seems unlikely for comparisons of locomotion in water and on land given the dramatically different physical characteristics of aquatic and terrestrial habitats (Biewener and Gillis, 1999; Gillis and Blob, 2001), and because such fixed motor patterns might actually impede performance of some behaviors (Biewener and Gillis, 1999; Blob et al., 2008). However, such motor stereotypy might be found if a central pattern generator were the dominant source of control for the muscles in question (Buford and Smith, 1990; Pratt et al., 1996; Blob et al., 2008), possibly simplifying locomotor control in systems with serially homologous appendages. A second possible strategy is that the same set of muscles might be recruited across behaviors, but with differences in timing or intensity of activity (Gruner and Altman, 1980; Roy et al., 1985; Macpherson, 1991; Roy et al., 1991; Johnston and Bekoff, 1996; Kamel et al., 1996; Gillis and Biewener, 2000; Reilly and Blob, 2003; Blob et al., 2008). Depending on the functional demands and requirements of the motion in question, some general patterns of coactivation may be maintained with only small differences in the intensity or timing of muscle activity (Gruner and Altman, 1980; Johnston and Bekoff, 1996). In other cases the timing of muscle activity might change so drastically between motor tasks that synergistic muscles in one task could act as antagonists in another (Buchanan et al., 1986). As a third possibility, different motor tasks might be accomplished through the actions of different muscles, or through the recruitment of specific muscles only during the performance of specific tasks (Gatesy, 1997). Because vertebrate limb musculature is highly redundant, with multiple muscles able to contribute to movement in each direction, these three possibilities are not mutually exclusive (Biewener and Gillis, 1999; Gillis and Blob, 2001; Blob et al., 2008). Several previous examinations of limb muscle motor patterns during aquatic versus terrestrial locomotion have found that modifications of at least some aspects of muscle activity are required to produce effective locomotion through both aquatic and terrestrial environments (Biewener and Gillis, 1999; Gillis and Biewener, 2001; Gillis and Blob, 2001; Blob et al., 2008). However, these studies, like the majority that have compared limb muscle motor patterns across disparate tasks (Ashley-Ross, 1995; Kamel et al., 1996; Ashley-Ross and Lauder, 1997; Gatesy, 1997; Gatesy, 1999; Gillis and Biewener, 2000; Gillis and Biewener, 2001; Higham and Jayne, 2004), have focused on the hindlimb. How similar are the modulation of fore- and hindlimb motor patterns across locomotor behaviors with different demands? Are modulation patterns observed in one set of limbs a good predictor of those in the other?

Turtles are an excellent group in which to examine questions about environmentally correlated modulation of motor patterns for several reasons. First, many species of turtles regularly perform both aquatic and terrestrial locomotion as part of their natural behaviors, with many species spending substantial amounts of time in both types of environments (Cagle, 1944; Bennett et al., 1970; Gibbons, 1970; Zug, 1971; Davenport et al., 1984; Ernst et al., 1994; Gillis and Blob, 2001; Blob et al., 2008). Second, because the rigid body design of turtles involves fusion of most of the body axis to a bony shell, propulsive forces are generated almost exclusively by the limbs in any habitat (Blob et al., 2008). Thus, evaluations of differences in limb muscle motor patterns across habitats will not be confounded by changes in the contribution of other structures to propulsion, like flexible bodies, tails, or specialized fins (Blake et al., 1995; Walker, 2000; Fish, 2002; Fish and Nicastro, 2003; Rivera et al., 2006). Additionally, because freshwater turtles (with the exception of the pig-nosed turtle, *Carettochelys insculpta*) use fore- and hindlimbs for locomotion it makes them ideal for studying both sets of limbs. While locomotor activity of the hindlimb muscles has been examined in two species of turtle, the slider (*Trachemys scripta*) and the spiny softshell (*Apalone spinifera*) (Gillis and Blob, 2001; Blob et al., 2008), the forelimb has not been examined.

In this study, I examined how muscle function is modulated to accommodate different performance demands by comparing the motor patterns of forelimb muscles in a generalized freshwater turtle, *Trachemys scripta* (Schoepff) (red-eared slider turtle), during aquatic and terrestrial locomotion. Like many freshwater turtles, sliders spend considerable time in the water, but also move over land to perform vital tasks such as nesting, basking, or moving between aquatic habitats (Gibbons, 1970; Gibbons, 1990; Ernst et al., 1994; Bodie and Semlitsch, 2000). Sliders must use the same set of muscles to produce these movements under the different performance demands of both habitats. These differing demands provide a basis for several predictions of how slider forelimb muscle activity might be modulated between water and land. First, because water is much more dense and viscous than air, turtles may show elevated activity in limb protractors during swimming versus walking in order to overcome the greater drag incurred during the recovery phase in water versus on land (Gillis and Blob, 2001). Conversely, the limb retractors may show elevated activity on land relative to water in order to counteract gravitational loads and support the body without the benefit of buoyancy (Gillis and Blob, 2001). Such differences in activity between habitats could be produced through changes in the duration of muscle bursts, the intensity of muscle activity, or both. Yet, though attractive to apply to the forelimb, EMG data from the hindlimb of *T. scripta* (and a second turtle species, the spiny softshell, *Apalone spinifera*) during swimming and walking do not uniformly support these predicted modulations of motor pattern based on differences in the physical characteristics of the locomotor environment (Gillis and Blob, 2001; Blob et al., 2008). For example, the mean amplitudes of bursts by two stance/thrust phase muscles, the hip retractor flexor tibialis internus (FTI) and the knee extensor femorotibialis (FT), are both greater in water than on land in *T. scripta* (Gillis and Blob, 2001; Blob et al., 2008). In addition, though one hindlimb protractor, iliofemoralis (ILF), showed bursts of greater mean amplitude, as predicted, during swimming compared to walking, a second hindlimb protractor with activity nearly synchronous with ILF, the puboischiofemoralis internus (PIFI), showed the opposite pattern of modulation, with higher amplitude bursts on land (Gillis and Blob, 2001; Blob et al., 2008). It is uncertain whether forelimb muscles should be expected to show patterns of modulation that follow predictions based on physical differences in locomotor environment, or whether they might show patterns similar to those of the serially homologous hindlimb. My EMG data from slider forelimbs will allow me to address this question, helping to build understanding of how animals modulate muscle activity to accommodate different environments and potentially contributing insights into how new forms of quadrupedal locomotion evolve.

Materials and Methods

Experimental animals

Slider turtles were purchased from a commercial vendor (Concordia Turtle Farm, Wildsville, LA, USA). Seven juvenile animals (four years old) that were similar in carapace length (average 14.5±0.6 cm) and body mass (average 450±42 g) contributed data to this study. Turtles were housed in groups in 600 liter (150 gallon) stock tanks equipped with pond filters and dry basking platforms. Tanks were located in a temperature-controlled greenhouse facility, thus exposing turtles to ambient light patterns during the course of experiments (February – May). Turtles were fed a diet of commercially available reptile food (ReptoMin®, Tetra®, Blacksburg, VA, USA), supplemented with earthworms. All animal care and experimental procedures were conducted in accordance with Clemson University IACUC guidelines (protocol 50110).

Collection and analysis of kinematic data

Kinematic data were collected simultaneously in lateral and ventral views (100 Hz) using two digitally synchronized high-speed video cameras (Phantom V4.1, Vision Research, Inc.; Wayne, NJ, USA). Locomotor trials (swimming and walking: Appendix A) were conducted in a custom-built recirculating flow tank with a transparent glass side and bottom. Ventral views were obtained by directing the ventral camera at a mirror oriented at a 45° angle to the transparent bottom of the tank. For aquatic trials, the tank was filled with water and flow was adjusted to elicit forward swimming behavior (Pace et al., 2001). Once the turtle was swimming, flow was adjusted to keep pace with the swimming speed of the animal. For terrestrial trials, water was drained from the tank, the glass was dried thoroughly, and turtles were encouraged to walk forward by gently tapping the back of the shell and providing them with a dark hiding spot at the far end of the tank. Although dried glass clearly differs from the substrate the turtles would encounter in nature, a transparent surface through which I could film was required. Because the glass and turtle were thoroughly dried prior to terrestrial trials the surface was not slippery, and all animals walked normally. Aquatic and terrestrial locomotor sequences were collected from each turtle, yielding 16-20 limb cycles for each habitat, from each turtle.

To facilitate digitization of animal movement from videos, a combination of white correction fluid and black marker pen were used to draw high-contrast points on the following 13 anatomical landmarks (Fig. 2.1): tip of the nose; shoulder; elbow; wrist; digits 1, 3, and 5; an anterior and posterior point on the bridge of the shell (visible in lateral and ventral view); and right, left, anterior, and posterior points on the plastron (plastral points visible in ventral view only). Landmark positions were digitized frame-by-frame in each video using DLTdataViewer2 (Hedrick, 2008). The three-dimensional coordinate data generated were then processed using custom Matlab (Student Ver. 7.1, MathWorks, Inc.; Natick, MA, USA) routines to calculate limb kinematics during swimming and walking, including protraction and retraction of the humerus, elevation and depression of the humerus, and extension and flexion of the elbow. Calculated values for kinematic variables from each limb cycle were fit to a quintic spline (Walker, 1998) to smooth the data, and interpolated to 101 values, representing 0 through 100 percent of the limb cycle. Transformation of the

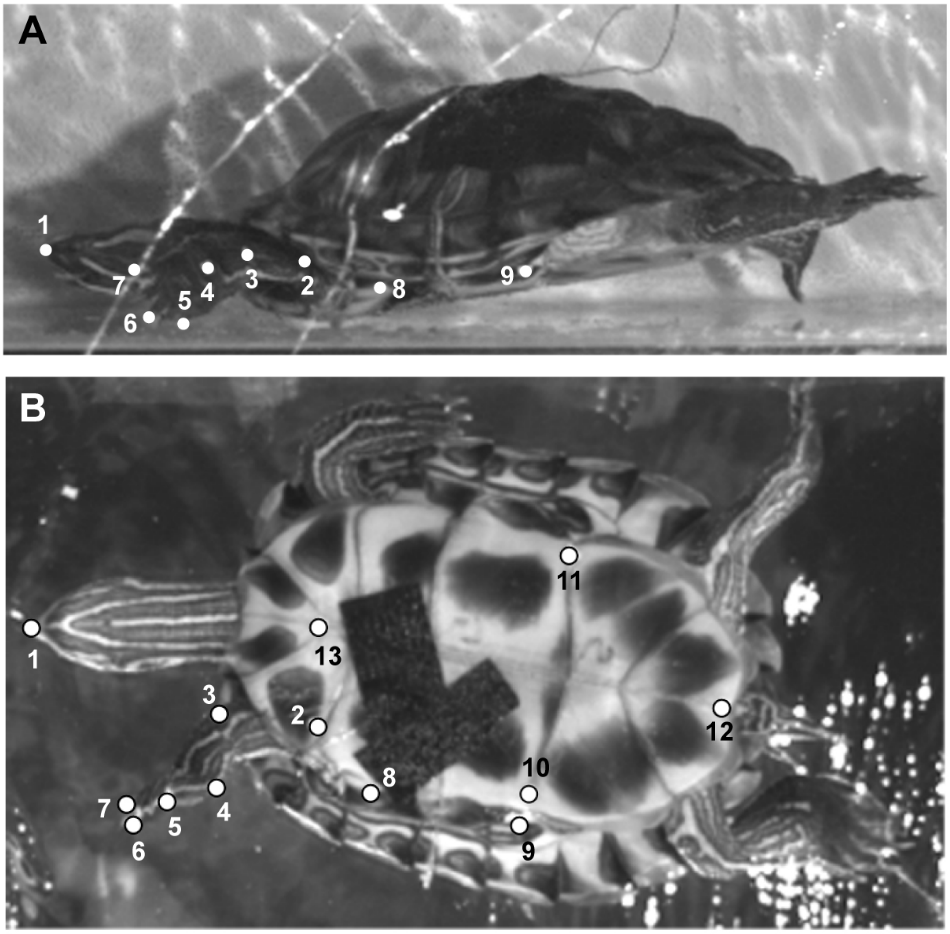

Fig. 2.1. Representative still images from lateral (A) and ventral (B) videos showing landmarks digitized for kinematic analysis. Points 1-9 are the same in lateral and ventral view; points 10-13 are only visible in ventral view. Landmarks include: 1- tip of the nose, 2- shoulder, 3- elbow, 4-wrist, 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, and 13-anterior point on plastron.

duration of each cycle to a percentage allowed me to compare locomotor cycles of different absolute durations and calculate average kinematic profiles and standard errors for each variable through the course of walking and swimming trials. A humeral protraction/retraction angle of 0° indicates that the humerus is perpendicular to the midline of the turtle, while an angle of 90° indicates a fully protracted forelimb with the distal end of the humerus directed anteriorly (an angle of -90° would indicate a fully retracted forelimb with the distal tip of the humerus directed posteriorly). A humeral elevation/depression angle of 0° indicates that the humerus is in the turtle's horizontal plane. Angles greater than zero indicate elevation above the horizontal (distal end above proximal end) while negative angles indicate depression of the humerus (distal end lower than proximal end). Extension of the elbow is indicated by larger extension/flexion angles and flexion is indicated by smaller values. An elbow angle of 0° indicates the hypothetical fully flexed (i.e., humerus perfectly parallel to radius and ulna) elbow, 180° indicates a fully extended elbow, and 90° indicates that the humerus is perpendicular to the radius and ulna. Forefoot orientation angle was also calculated as the angle between a vector pointing forwards along the anteroposterior midline (also the path of travel) and a vector emerging from the palmar surface of a plane defined by the tips of digits 1 and 5 and the wrist; this angle was transformed by subtracting 90° from each value (Pace et al., 2001). A high-drag orientation of the forefoot paddle with the palmar surface of the paddle directed opposite the direction of travel (and in the same direction as the flow of water) is indicated by an angle of 90°, and a perfect low-drag orientation of the forefoot paddle is indicated by an angle of 0°.

Kinematics were tested at speeds chosen by the animals (Pace et al., 2001) which, for terrestrial locomotion in particular, were difficult to control. Additionally, freshwater turtles typically swim faster than they walk (Blob et al., 2008). Because I sought to compare motor patterns for typical swimming and walking behaviors, I therefore collected data over a range of speeds for both behaviors. Swimming *T. scripta* completed limb cycles in 0.46±0.01 s (mean ± S. E. M.), whereas walking limb cycle durations averaged 1.03±0.04 s. While there was greater variability in the time required to complete walking cycles (0.36-2.88 seconds) versus swimming cycles (0.25-0.80 seconds) these ranges showed extensive overlap. No differences in kinematics (or muscle activity) were evident across the relatively broader range of speeds exhibited during walking.

Collection and analysis of electromyographic data

Concurrent with video acquisition, electromyography (EMG) was used to measure muscle firing patterns of target forelimb muscles (Loeb and Gans, 1986). Following previously established protocols (Loeb and Gans, 1986; Westneat and Walker, 1997; Gillis and Blob, 2001; Blob et al., 2008), turtles were anesthetized with intramuscular injections of ketamine HCl (90-100 mg/kg) 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 forelimb using hypodermic needles. External landmarks for implants were determined prior to experiments through dissection, helping to ensure accurate placement of electrodes. Up to 12 implants were performed for each experiment, with target muscles receiving multiple electrodes (typically 2 or 3, but occasionally up to 4) to help ensure successful recordings even if some electrodes failed. Electrode wires exiting the forelimb were allowed several centimeters of slack before being bundled together and glued into two separate cables that were directed ventrally and posteriorly to run along a segment of the plastron, and then dorsally along the curve of the bridge before being secured to the carapace using waterproof tape (Fig. 2.1). The anterior cable bundle contained electrodes from the medial side of the forelimb, and the posterior cable contained electrodes from the lateral side. Following electrode implantation, the locations of digitizing landmarks were marked (as described above) and turtles were allowed to recover overnight. During locomotor trials, EMG signals were relayed from the electrodes in each turtle to a Grass 15LT amplifier system (West Warwick, RI, USA) for amplification (usually 10,000 times, but occasionally set to 5,000 times) and filtering (60Hz notch filter, 30Hz-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. Kinematic data were synchronized with electromyographic data by triggering a signal generator that simultaneously produced a light pulse visible in the video and a square wave in the EMG data. Following data collection, turtles were euthanized via intraperitoneal injection of sodium pentobarbital (200 mg/kg) and electrode positions were verified by dissection.

I focused on five target muscles for this study, covering all major planes of motion of the forelimb during swimming and walking (Fig. 2.2). Predicted actions for each muscle were based on anatomical position (Walker, 1973). The coracobrachialis is positioned posterior to the humerus and expected to retract the forelimb. The pectoralis is a large, triangular sheet that extends widely from approximately the plastral midline to converge and insert on the flexor border of the lateral process of the humerus, and is predicted to retract and depress the humerus. Latissimus dorsi is positioned anterior and dorsal to the humerus and is predicted to protract and elevate the limb. The deltoid is located more ventrally, attaching to the plastron close to its midline and running to the shoulder joint, but also with predicted actions of humerus protraction and elevation. Finally, the triceps complex is located on the extensor surface of the arm, running from the shoulder joint to the elbow, and is predicted to act in elbow extension. Data were incidentally collected from two additional muscles: supracoracoideus, a large ventral muscle deep to the pectoralis with anterior and posterior heads, is predicted to retract and depress the humerus [though some anterior fibers might aid protraction (Walker, 1973)]; and the subscapularis, the largest dorsal muscle on the pectoral girdle, covering the lateral, posterior, and much of the medial surface of the scapula and predicted to elevate the humerus. The subscapularis was sampled using two different approaches; in a “cor approach” the electrode

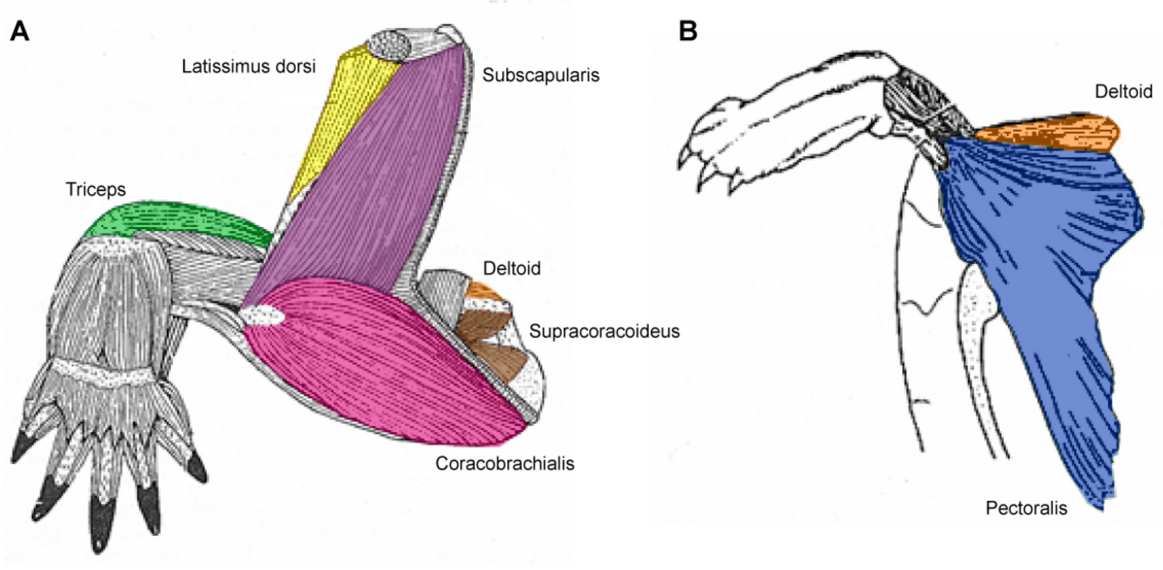

Fig. 2.2. Illustration showing the five target muscles and two supplemental muscles from which electromyographic data were collected. (A) Posterior view of the left forelimb musculature of *Trachemys scripta*; modified from Walker (1973). (B) Ventral view of the forelimb musculature of *Trachemys scripta*; modified from Wyneken (1997). Predicted muscle actions are based on their anatomical positions. Coracobrachialis (pink) is situated posterior to the humerus and expected to retract the forelimb. The most ventral target muscle, pectoralis (blue) extends from the plastral midline towards the anterior margin of the bridge to a tendon that inserts on the lateral process of the humerus, and is predicted to retract and depress the humerus. Latissimus dorsi (yellow) is anterior and dorsal to the humerus and is predicted to protract and elevate the forelimb. More ventrally is the deltoid (orange), which runs from the plastron to the shoulder joint and is predicted to protract and elevate the humerus. Triceps (green) is located on the extensor surface of the arm, running from the shoulder joint distally to the elbow, and is predicted to act in elbow extension. Subscapularis (purple) is the largest of the dorsal pectoral girdle muscles, occupying much of the posterior, lateral, and medial surfaces of the scapular prong, and is predicted to elevate the humerus. Supracoracoideus (brown) is deep to pectoralis, divided into anterior and posterior heads, and predicted to retract the humerus.

was implanted into the muscle by inserting it more posteriorly and laterally (as if approaching coracobrachialis), whereas in a “lat approach” the electrode was implanted into the muscle by aiming more anteriorly (as if approaching latissimus dorsi). These two approaches, and therefore separate segments of muscle, are henceforth, referred to as subscapularis (cor approach) and subscapularis (lat approach).

EMG data were analyzed using custom LabVIEW software routines to identify bursts of muscle activity. EMG variables calculated included onset, offset, and duration of muscle bursts, as well as mean amplitude of each burst (to provide a measure of intensity). The mean amplitude recorded from different electrodes should not be compared because minor differences in electrode construction can affect signal strength (Loeb and Gans, 1986). For this reason, burst intensities were normalized for each electrode by dividing the mean amplitude for each burst by the maximum value for mean amplitude recorded from that electrode throughout aquatic and terrestrial trials (Gillis and Biewener, 2000; Konow and Sanford, 2008). This enables the comparison of burst intensity across individuals, allowing me to determine if there are consistent patterns of intensity change between swimming and walking.

Statistical analysis

To assess general patterns of movement and muscle function, the overall mean and standard error of each variable was calculated for all terrestrial and aquatic trials. Muscle activity variables include for each muscle: (i) onset, (ii) offset, (iii) duration, and (iv) normalized mean amplitude. Kinematic variables include: (i) maximum protraction, retraction, elevation, and depression of the humerus, (ii) maximum elbow extension and flexion, (iii) anteroposterior and dorsoventral excursion of the humerus, (iv) elbow excursion, (v) percentage of the cycle at which maximum elbow extension occurs, (vi) the percentage of the limb cycle at which a switch from protraction to retraction occurs, and (vii) the degree of feathering of the forefoot during protraction. Because the maximum values for each limb cycle do not always occur at the same percentage of the limb cycle, it is possible that the average of the maximum values calculated for all limb cycles may be masked (appear lower) in average kinematic profiles. I used Systat (v.12) for all statistical analyses, and $P<0.05$ as the criterion for significance.

To determine the effect of environment on variables characterizing forelimb kinematics and muscle function, I conducted two-way, mixed-model analyses of variance (ANOVA), with environment as a fixed factor and individual as a random factor. Two-way mixed model ANOVAs (corrected for unbalanced sampling) were performed separately on each variable, except for the coracobrachialis, the supracoracoideus (anterior head), and the subscapularis (lat approach), which were sampled in an insufficient number of individuals, or incompletely within individuals, and which were, therefore, analyzed separately using one-way ANOVAs with habitat as the independent factor and values for each habitat pooled together. Two-way mixed model ANOVAs were calculated using individual variation as the error term, whereas one-way ANOVAs were calculated using cycle to cycle variation as the error term. One set of ANOVAs was performed on data from each muscle and on each kinematic variable; kinematic and timing variables include data from all recordings, but intensity comparisons only include data from individuals for which I successfully recorded both swimming and walking from the same electrode. In tabular data summaries I provide degrees of freedom and *F*-values, in addition to results of sequential Bonferroni corrections (Holm, 1979; Rice, 1989), to clarify the potential effects of making multiple comparisons. For statistical analyses of EMG timing variables (onset, offset, duration), only data from individuals with both aquatic and terrestrial EMG data were used (see Appendix B). For statistical analyses of EMG intensity variables, only data from individuals in which the same electrode successfully recorded during both aquatic and terrestrial trials were used (see Appendix B).

Results

For kinematic analyses, 16-20 swimming and walking trials were obtained from each of six turtles, with a seventh providing a similar number of swimming trials but fewer walking trials (see Appendix A). The number of trials from which EMG data were collected is variable across individuals and muscles due to differences in the success of electrode implants. Plots depicting the general pattern of muscle activation during swimming and walking were constructed using all collected and verified EMG data (see Appendix C). A general summary of sample sizes from each individual, and from each environmental condition, are given for statistical analyses (see Appendices A, B) and EMG timing variables (see Appendix C).

Kinematics of swimming and walking

Previously published descriptions of forelimb kinematics in swimming *T. scripta* (in the context of a comparison to an aquatic specialist *Apalone spinifera*, Pace et al., 2001) were for larger individuals than those used in this study; I describe aquatic forelimb kinematics here with a focus on comparison with terrestrial kinematics and synchronization with EMG data. For both swimming and walking, the limb cycle is defined as starting at the beginning of humeral protraction and ending at the start of the next protraction cycle. The limb cycle can be divided into two separate phases; humeral protraction represents the “recovery” phase in water or the “swing” phase on land, followed by retraction of the humerus through the “thrust” phase in water or the “stance” phase on land.

In both aquatic and terrestrial locomotion there is a single peak of humeral protraction. The duration of protraction differs significantly between swimming and walking, with protraction comprising the first 43±0.6 % (mean ± S. E. M.) of the limb cycle in swimming, and only the first 21±0.6 % of the cycle during walking (Fig. 2.3A, Table 2.1). The humerus is protracted significantly more

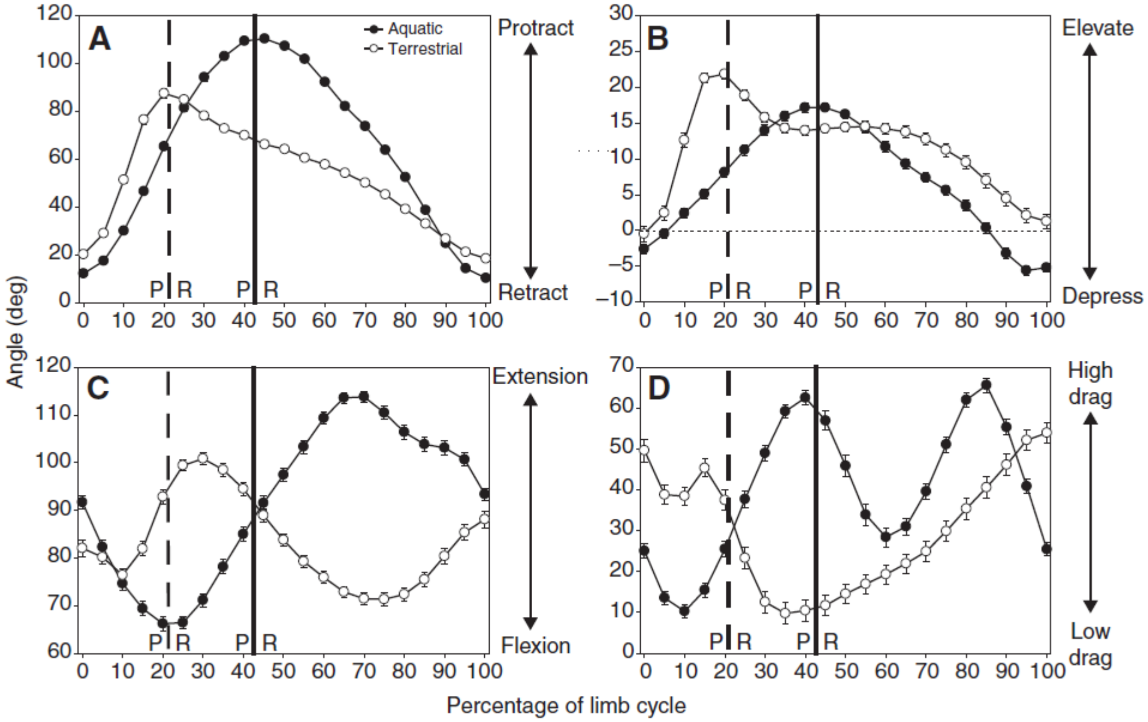

Fig. 2.3. Mean kinematic profiles for *Trachemys scripta* during swimming (filled symbols) and walking (open symbols). Each trial was normalized to the same duration and angle values interpolated to 101 points representing 0-100% of the limb cycle. The limb cycle is defined as protraction of the humerus followed by retraction. Mean angle values ± S.E.M. are plotted for every fifth increment (every 5% through the cycle) for all individuals. Vertical lines demarcate the switch from protraction (P) to retraction (R) for swimming (solid) and walking (dashed). (A) Humeral protraction and retraction (i.e., angle from the transverse plane). An angle of 0° indicates that the humerus is perpendicular to the midline of the turtle, while an angle of 90° indicates a fully protracted forelimb with the distal end of the humerus directed anteriorly (an angle of -90° would indicate a fully retracted forelimb with the distal tip of the humerus directed posteriorly). (B) Humeral elevation and depression (i.e., angle from the horizontal plane). An angle of 0° indicates that the humerus is in the horizontal plane. Angles greater than zero indicate elevation above the horizontal (distal end above proximal end) and negative angles indicate depression of the humerus (distal end lower than proximal end). Peak elevation is coincident with peak protraction for both swimming and walking, meaning that limb protraction happens at the same time as elevation and retraction is concurrent with depression. (C) Elbow flexion and extension. Extension is indicated by larger angles and flexion is indicated by smaller angles. An angle of 0° indicates complete flexion, 180° indicates a fully extended elbow, and 90° indicates that the humerus is perpendicular to the radius and ulna. (D) Forefoot orientation angle is calculated as the angle

between a vector pointing forwards along the anteroposterior midline (also the path of travel) and a vector emerging from the palmar surface of a plane defined by the tips of digits 1 and 5 and the wrist; this angle is transformed by subtracting 90° from each value. A high-drag orientation of the forefoot paddle with the palmar surface of the paddle directed opposite the direction of travel (and in the same direction as the flow of water) is indicated by a feathering angle of 90°, and a perfect low-drag orientation of the forefoot paddle is indicated by a feathering angle of 0°. Feathering of the forefoot paddle during retraction is obscured during walking because the foot is on the substrate and the limb is supporting the body.

during swimming (115±1.4°) than in walking (99±1.9°), though both locomotor behaviors are characterized by roughly similar humeral retraction (Fig. 2.3A). Total anteroposterior excursion of the humerus also differs significantly between the two environments, with the humerus experiencing a much larger range of motion during swimming (107±1.7°) than during walking (85±2.3°)(Table 2.1, Fig. 2.3A).

Peak humeral elevation (Fig. 2.3B) differs significantly between swimming (20±0.7°) and walking (26±0.6°, Table 2.1), and is roughly coincident with the switch from protraction to retraction (Table 2.1, Fig. 2.3A), indicating that the limb reaches maximum elevation in both swimming and walking at or near the end of recovery/swing phase. The humerus is greatly elevated during the recovery phase (i.e., swing phase; Fig. 2.3B) of walking as the limb is swung up and forward (Fig. 2.3A, B). Elevation of the humerus during the recovery phase of swimming is more gradual than that during the swing phase of walking (Fig. 2.3A,

Table 2.1. Mean values and standard errors of humeral kinematic variables and F-values for the main effect of habitat from two-way mixed model ANOVAs performed separately on each variable

Variable	Aquatic	Terrestrial	*F*-value (d.f. 1,6)
Maximum humeral retraction[1]	8±0.8	14±1.0	4.8
Maximum humeral protraction[1]	115±1.4	99±1.9	13.4**
% of limb cycle at maximum protraction[2]	43±0.6	21±0.6	331.4***†
Anteroposterior humeral excursion angle[3]	107±1.7	85±2.3	14.8**
Maximum humeral depression[1]	-8±0.6	-4±0.9	1.5
Maximum humeral elevation[1]	20±0.7	26±0.6	6.7*
Dorsoventral humeral excursion angle[3]	28±0.7	30±1.0	0.4
Maximum elbow flexion[1]	61±1.3	61±0.9	0.1
Maximum elbow extension[1]	123±0.9	113±1.2	9.3*
% of limb cycle at maximum elbow ext.[2]	68±1.3	36±2.4	31.6***†
Elbow excursion angle[3]	62±1.5	52±1.1	1.3
Forefoot feathering excursion (protraction)[3]	65±1.3	46±1.9	18.2**†

[1] Values are angles in degrees
[2] Values represent a percentage of the limb cycle
[3] Values represent the total angular excursion

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$
† Significant after sequential Bonferroni correction

B). In both swimming and walking, the limb reaches its greatest anterior extent and elevation just prior to the beginning of retraction. At this point, the extreme angle of protraction of the humerus (115±1.4° for swim ming and 99±1.9° for walking), shifts the position of the elbow medial to the shoulder and above the head [a result also found by Pace et al. (2001) for swimming]. Maximum humeral depression and dorsoventral excursion of the humerus do not differ significantly between swimming and walking (Table 2.1). During retraction, the humerus is depressed while it is moved posteriorly until maximal retraction and depression are reached nearly simultaneously (Fig. 2.3A, B).

Elbow extension patterns differed between swimming and walking (Fig. 2.3C). During swimming, *T. scripta* flex the elbow for the first half of protraction and then begin elbow extension, reaching maximum extension midway through retraction, and then flexing the elbow for the remainder of the limb cycle to return to the starting position (Fig. 2.3C). During walking, as in swimming, the elbow is flexed until midway through protraction when extension begins (Fig. 2.3C). However, unlike swimming, maximum elbow extension is reached very early during terrestrial retraction, followed quickly by a period of elbow flexion as the limb begins to support the weight of the body, and a second phase of elbow extension follows as the body is propelled anteriorly relative to the supporting limb (Fig. 2.3C). While maximum elbow flexion and excursion did not differ between swimming and walking, maximum elbow extension was significantly greater in swimming than in walking (123±0.9° versus 11 3±1.2°; Table 2.1) and occurred significantly later in the limb cycle (68±1.3% swimming versus 36±2.4% walking; Table 2.1).

The orientation of the forefoot relative to the direction of travel (or the direction of water flow) differs between swimming and walking (Fig. 2.3D). In water, this variable indicates whether the forefoot is in a high drag orientation with the plane of the forefoot perpendicular to the direction of travel, or a low drag (feathered) orientation (Pace et al., 2001). Similar to results from Pace et al. (2001), the forefoot of *T. scripta* is feathered in a low-drag orientation early in protraction and reaches a first peak of high-drag orientation (nearly perpendicular to the flow of water) very near the end of protraction; this is followed by a second, high-drag peak at roughly two-thirds through the retraction phase, and ends with the palmar surface of the forefoot directed dorsally (Fig. 2.3D). During the protraction phase of walking, the forefoot is held in a less feathered orientation than in swimming, and the total feathering excursion angle experienced by the forefoot during protraction is significantly greater during swimming than walking (65±1.3° versus 46±1.9°; Fig. 2.3D; Table 2.1). During the stance phase of walking, the forefoot is placed flat relative to the ground, as it must support the weight of the body, but then gradually peels off the substrate to an angle more perpendicular to the ground.

In summary, though both swimming and walking are characterized by the same general motions of the forelimbs in *T. scripta*, there are several striking differences (Table 2.1). The timing of protraction and retraction differs greatly between swimming and walking, as does the maximum angle of humeral protraction and the anteroposterior excursion angle of the humerus, though the humerus is retracted to nearly the same degree in both environments. Peak elevation of the humerus is coincident with peak protraction in both environments, but while there is significantly greater elevation during walking, the level of humeral depression does not differ between habitats. The elbow is held straighter during walking, but with peak extension occurring significantly later in the limb cycle than during swimming. Finally, during protraction, sliders showed a much greater angular excursion range for orientation of the forefoot during swimming versus walking.

Patterns of muscle activation during swimming and walking

Among predicted humeral retractors, coracobrachialis exhibits a single burst of activity during most of retraction phase in both swimming and walking, though onset, offset, and duration of activity relative to the entire limb cycle differ significantly between environments for this muscle (Fig. 2.4, Table 2.2). In contrast, the other predicted humeral retractor, pectoralis, exhibits two bursts of activity in swimming but only one during walking (Fig. 2.4). The early burst of activity seen in pectoralis during swimming is variable, in that it was not present in every swimming cycle; two of five turtles never showed this early burst, one individual (TS09) always did, another did most of the time (TS11, 18 of 20), and the final turtle (TS99) seldom did (2 of 20 cycles). Verification dissections revealed no detectable differences in placement of the electrodes across turtles that varied with regard to the presence of this variable burst, and kinematics did not clearly differ in relation to whether the burst was present or absent. This early variable burst of pectoralis activity during swimming occurs fully during protraction when present, whereas the later burst of activity for pectoralis that

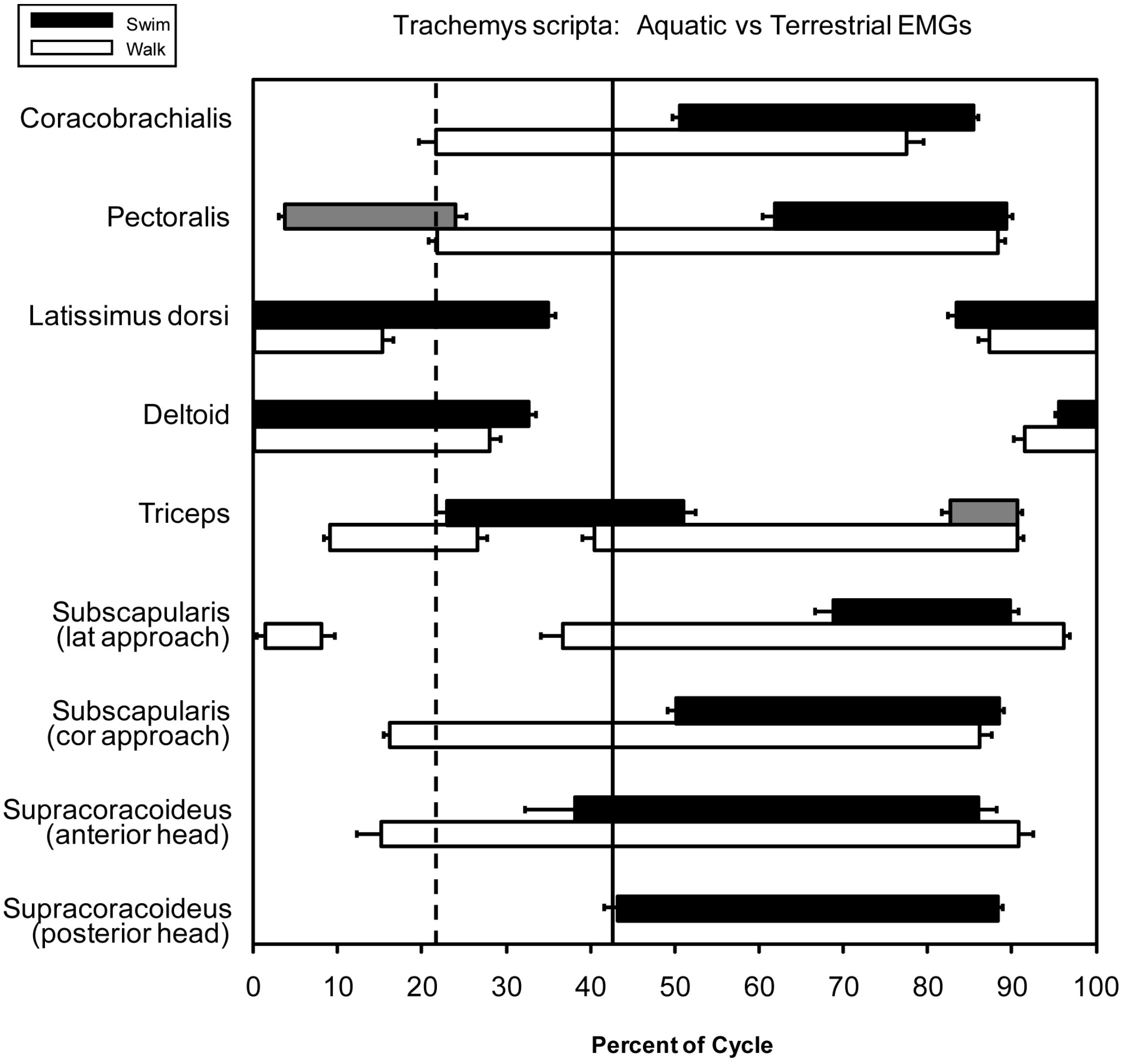

Fig. 2.4. Bar plot showing patterns of forelimb muscle activation during swimming and walking in *Trachemys scripta*. Bars represent the mean and standard error for the period of activity for each muscle. Solid bars represent swimming, open bars represent walking, and gray bars represent variable bursts of muscle activity observed during swimming that were not always present. Vertical lines demarcate the switch from protraction to retraction for walking (dashed line) and swimming (solid line). The x-axis shows the percent of the limb cycle from 0 to 100%. “Cor approach” indicates that the electrode was implanted into the muscle by inserting it more posterior and laterally (as if approaching coracobrachialis). “Lat approach” indicates the electrode was implanted into the muscle by inserting it more anteriorly (as if approaching latissimus dorsi). Note that data from the posterior head of the supracoracoideus were only obtained during swimming; this does not, however, indicate that there was no activity during walking.

Table 2.2. Mean values and standard errors for EMG timing and amplitude variables and F-values for the main effect of habitat

Variable	Swimming	Walking	F-value	d.f.
<u>Coracobrachialis</u>				
Onset	51±1	22±2	205.43***†	1,106
Offset	85±0.5	77±2	31.99***†	1,106
Duration	34.9±1.2	56±2.3	78.55***†	1,106
Normalized Amplitude	0.43±0.04	0.2±0.003	5.35*†	1,22
<u>Pectoralis Burst #1</u>[1]				
Onset	4±0.7	22±1	118.89***†	1,2
Offset	24±1.3	88±0.9	146.18**†	1,2
Duration	20±1.7	67±1.1	41.87*	1,2
Normalized Amplitude	0.36±0.03	0.45±0.02	0.02	1,2
<u>Pectoralis Burst #2</u>[1]				
Onset	62±1.5	22±1.0	27.44**†	1,4
Offset	89±0.7	88±0.9	0.01	1,4
Duration	28±1.3	67±1.1	46.16**†	1,4
Normalized Amplitude	0.55±0.03	0.45±0.02	1.47	1,4
<u>Latissimus dorsi "Burst #1"</u>				
Offset	35±0.9	15±1.2	23.59*	1,2
Duration	35±0.9	14±1.1	39.26*	1,2
Normalized Amplitude	0.44±0.03	0.33±0.04	0.18	1,2
<u>Latissimus dorsi "Burst #2"</u>				
Onset	83±1	87±1.2	0.70	1,2
Duration	16±1	13±1.1	0.73	1,2
Normalized Amplitude	0.4±0.03	0.2±0.02	3.55	1,2
<u>Deltoid "Burst #1"</u>				
Offset	34±1.0	28±1.4	2.06	1,4
Duration	32±1.2	25±1.1	3.86	1,4
Normalized Amplitude	0.34±0.02	0.29±0.03	0.34	1,4

Table 2.2., continued

Variable	Swimming	Walking	F-value	d.f.
Deltoid "Burst #2"				
Onset	96±0.4	91±1.3	3.96	1,3
Duration	4±0.4	8±1.2	3.53	1,3
Normalized Amplitude	0.4±0.04	0.13±0.02	2.09	1,3
Triceps Burst #1				
Onset	23±1.3	9±0.7	4.49	1,4
Offset	51±1.5	26±1.5	7.92*	1,4
Duration	28±0.9	18±1.5	2.36	1,4
Normalized Amplitude	0.49±0.02	0.38±0.03	0.009	1,3
Triceps Burst #2				
Onset	83±1	39±1.7	49.92**†	1,4
Offset	91±0.6	92±0.6	0.60	1,4
Duration	8±0.5	54±1.8	84.36***†	1,4
Normalized Amplitude	0.5±0.03	0.33±0.02	0.27	1,3
Subscapularis (lat approach) Burst #1				
Onset	-----	1±1.1	-----	-----
Offset	-----	8±1.6	-----	-----
Duration	-----	7±0.8	-----	-----
Normalized Amplitude	-----	0.30±0.02	-----	-----
Subscapularis (lat approach) Burst #2				
Onset	69±2.1	37±2.7	88.91***†	1,35
Offset	90±0.9	96±0.8	25.41***†	1,35
Duration	21±2.1	59±3.2	105.07***†	1,35
Normalized Amplitude	0.7±0.04	0.44±0.04	24.65***†	1,35
Subscapularis (cor approach)				
Onset	50±1	16±0.7	32.79	1,1
Offset	88±0.5	86±1.5	0.11	1,1
Duration	38±1.3	70±1.4	13.69	1,1
Normalized Amplitude	0.62±0.03	0.36±0.01	58.26	1,1

Table 2.2., continued

Variable	Swimming	Walking	F-value	d.f.
<u>Supracoracoideus (anterior head) (TS14 only)</u>				
Onset	38±6	15±3	5.27*	1,28
Offset	86±2	91±1.6	2.38	1,28
Duration	48±5.9	76±2.9	8.64**†	1,28
Normalized Amplitude	0.36±0.09	0.1±0.02	5.38*	1,28
<u>Supracoracoideus (posterior head)</u>				
Onset	43±1.7	-----	-----	-----
Offset	88±0.6	-----	-----	-----
Duration	45±1.8	-----	-----	-----

cor approach = the electrode was implanted into the muscle by inserting it more posterior and laterally (as if approaching coracobrachialis); lat approach = the electrode was implanted into the muscle by inserting it more anteriorly (as if approaching latissimus dorsi)

Two-way mixed model ANOVAs performed separately on each variable, except for coracobrachialis, supracoracoideus (anterior head), and subscapularis (lat approach) which were analyzed separately with one-way ANOVAs. Amplitude comparison for coracobrachialis is for TS36 only.

[1] Aquatic EMGs for pectoralis showed an extra early burst of activity, whereas terrestrial EMGs never did. Because the "typical" pectoralis burst was later in the limb cycle, it is coded as Burst #2 even if there was only a single burst. Because terrestrial EMGs only ever showed a single burst, statistics were run in two ways: Aquatic Burst #1 vs Terrestrial Burst and Aquatic Burst #2 vs Terrestrial Burst.

"Burst #1" and "Burst #2" are used to indicate the early and late activity, respectively, of a muscle exhibiting a continuous burst of activity that spans the retraction to protraction phase shift. These muscles include deltoid and latissimus dorsi.

----- indicates that no data exist for this muscle burst so statistics were not necessary

Values are means ± standard error

*P≤0.05; **P≤0.01; ***P≤0.001

Sequential Bonferroni correction conducted for each muscle to assess the effects of multiple comparisons

† Significant after sequential Bonferroni correction

was always present occurred nearly entirely during retraction in both environments. Because there is only one burst of activity in walking, this single burst was compared to both bursts of activity seen during swimming (Table 2.2). Comparison to the early burst seen in swimming shows significant differences for onset, duration, and offset (Table 2.2) while comparison to the later burst during swimming shows significant differences in onset and duration, but not offset (Table 2.2).

Among humeral protractors, latissimus dorsi and deltoid both show one long continuous burst of activity in both environments, starting shortly before the end of retraction and continuing into the protraction phase (Fig. 2.4). Because my definition of the limb cycle divides these continuous bursts into two portions for graphic presentation, I use quotation marks to distinguish references to the “early” and “late bursts” (or “Burst 1” and “Burst 2”) for these muscles, in contrast to references to separate, non-continuous bursts of activity in other muscles. Thus, for latissimus dorsi and deltoid, onset refers to the beginning of activity observed for “Burst 2” and offset refers to the end of activity observed for “Burst 1”. The onset of “Burst 1” and the offset of “Burst 2” always occur at 0% and 100% of the limb cycle, respectively. Offset and duration differ significantly between swimming and walking for latissimus dorsi “Burst 1”, with activity ceasing later (and duration longer) in swimming; however, there were no differences between environment in the onset or duration of “Burst 2” (Fig. 2.4,

Table 2.2). Unlike latissimus, timing variables did not differ significantly between swimming and walking for either the “early” or “late” deltoid “bursts”.

Triceps is characterized by two bursts for both swimming and walking; one burst straddling the switch from protraction to retraction and the other occurring during the retraction phase of the limb cycle (Fig. 2.4). The later triceps burst was always present during walking, but was variably present during swimming (Fig. 2.4), always occurring in two turtles (TS02 and TS99) and in between 50 and 75 percent of cycles in the remaining three (11 of 20 for TS11, 10 of 20 for TS14, and 15 of 20 for TS31) (see Appendix C). Offset of Burst 1 of triceps activity occurs significantly later during swimming, with no significant differences in onset or duration of Burst 1 triceps activity, though, the timing of onset is visibly later during swimming (Fig. 2.4, Table 2.2). During swimming, onset of triceps Burst 2 occurs significantly earlier, and therefore duration is significantly longer; offset does not differ between habitats (Fig. 2.4, Table 2.2).

Among incidentally sampled muscles, subscapularis activity was recorded using electrodes implanted from two different approaches. The more posterior (cor approach) of subscapularis exhibits a single burst of activity for both swimming and walking, occurring mostly during retraction (Fig. 2.4). While the offset of activity is not significantly different, the duration of activity is significantly longer during walking, with onset occurring visibly (but not significantly) earlier in the limb cycle (Table 2.2). The more anterior (lat approach) implantation of subscapularis shows differing patterns, with two bursts of activity during walking and only one during swimming (Fig. 2.4). The early burst of subscapularis (lat approach) during walking occurs early in the protraction phase (Fig. 2.4). The second burst of walking subscapularis (lat approach) activity and the single swimming burst occur during retraction, with the walking burst starting significantly earlier and ending significantly later (Fig. 2.4, Table 2.2). The anterior head of supracoracoideus presents a single burst of activity in both swimming and walking, beginning just before the switch from protraction to retraction and lasting for most of retraction. While the offset of activity for this muscle did not differ between environments, onset occurs significantly earlier in walking, resulting in a significantly longer duration (Fig. 2.4, Table 2.2). The posterior head of the supracoracoideus was only sampled successfully during swimming, during which it showed one burst of activity starting just prior to, and continuing through, most of the retraction phase (Fig. 2.4).

Comparisons of the intensity of muscle activity (normalized mean amplitude) between habitats for pectoralis (each aquatic burst versus the terrestrial burst), latissimus dorsi and deltoid (both “early” and “late bursts” of activity), triceps, and subscapularis (cor approach) indicated no significant differences between water and land (Table 2.2). In contrast, swimming was characterized by greater intensity bursts for coracobrachialis, subscapularis (lat approach), and supracoracoideus (anterior head) (Table 2.2).

In cases where two bursts of activity were present for a muscle I tested for differences in intensity (Table 2.3). Two-way mixed-model ANOVAs detected no significant differences between bursts for deltoid, latissimus dorsi, pectoralis, or triceps during swimming or for latissimus dorsi or triceps during walking. The early period of deltoid activity during walking showed significantly higher mean amplitude than the later period (Tables 2.2, 2.3).

Table 2.3. Comparison of normalized EMG amplitude between multiple bursts with mean values, standard errors, F-values, p-values, and d.f. for the main effect of burst in two-way mixed model ANOVAs corrected for unbalanced sampling

Variable	Burst #1	Burst #2	F-value	p-value	d.f.
Aquatic					
Pectoralis	0.36±0.03	0.56±0.03	0.40	0.59	1,2
Latissimus dorsi	0.57±0.03	0.53±0.03	4.07	0.18	1,2
Deltoid	0.51±0.02	0.49±0.04	0.008	0.93	1,3
Triceps	0.54±0.02	0.56±0.03	0.01	0.92	1,4
Terrestrial					
Latissimus dorsi	0.36±0.04	0.22±0.03	2.18	0.26	1,2
Deltoid	0.42±0.03	0.24±0.03	48.32	0.0001	1,3
Triceps	0.53±0.03	0.51±0.03	0.34	0.60	1,3

Amplitude normalized separately for each habitat

Discussion

I identified several differences in the kinematics of swimming and walking in *Trachemys scripta*, including a longer duration of protraction, greater maximum humeral protraction, less humeral elevation, and a feathered forefoot orientation during the protraction phase of swimming. While most muscles examined were active when I predicted they would be, triceps, pectoralis, and subscapularis all showed additional bursts of activity. Contrary to predictions, I found no difference in the intensity of protractor activity during swimming versus walking and several retractors actually exhibited higher intensity bursts during swimming. Motor patterns for forelimb protractors are not consistent with those observed in functionally analogous hindlimb muscles, but motor pattern modulations for forelimb retractors between water and land are largely parallel between the fore- and hindlimb.

Kinematic comparison of swimming and walking

Several key differences emerge in the forelimb kinematics of *T. scripta* between aquatic and terrestrial locomotion. First, the protraction (or recovery) phase during swimming lasts almost twice as long as swing phase during walking (43±0.6% versus 21±0.6% of the limb cycle). This means that roughly equal time is spent in recovery and thrust phase in swimming, but only about a fifth of the limb cycle is spent during swing in walking. With regard to angular excursions, a general pattern that emerges is that one extreme of a range of motion differs between environments but the other does not. For example, maximum humeral retraction does not differ between swimming and walking, but the forelimb is protracted significantly more during swimming, resulting in vastly different ranges of anteroposterior humeral excursion between the two behaviors (Fig. 2.3A, Table 2.1). Similar maximal retractions between habitats could reflect a limit to the amount of retraction that is possible for the humerus of *T. scripta* due to the presence of the bridge of the shell posterior to the shoulder. In contrast, greater protraction of the forelimb during swimming would allow greater posterior excursion of the forelimb during retraction relative to that during walking, a pattern that might affect aquatic thrust production (Pace et al., 2001), though specific functional benefits to such differences in motion patterns between habitats remain to be tested. Maximal humeral depression is also similar during swimming and walking, but the swing phase of walking is characterized by a much greater maximum elevation angle than the recovery phase of swimming (Fig. 2.3B, Table 2.1). This distinction also might reflect the different demands placed on the musculoskeletal system between aquatic and terrestrial locomotion. Because turtle limbs need to clear the substrate during swing phase on land, substantial humeral elevation might be needed during walking. However, in freshwater turtles, forward thrust during swimming is generated primarily through anteroposterior movements of the limbs, so extraneous dorsoventral motions might be detrimental to thrust production and would be expected to be limited (Pace et al., 2001).

Elbow kinematics also differ between swimming and walking (Fig. 2.3C). During swimming, the elbow flexes for the first half of protraction as the forelimb moves towards the level of the shoulder, then extends through the remainder of protraction until about halfway through humeral retraction (i.e., thrust phase), when the elbow starts to flex again to move the forelimb paddle through the greatest arc possible to generate thrust for swimming. During walking, the elbow is also flexed for the first half of protraction, until the forelimb is moved to the level of the shoulder. However, the elbow then extends only until it reaches a maximum shortly after the start of the retraction phase, during which a second flexion-extension cycle is performed as the limb receives the weight of the body and pushes off to complete the step. As in movements at the shoulder, only one extreme of the range of elbow motion differs between swimming and walking. Maximum elbow flexion is almost identical between the two behaviors (61±1.3° in swimming versus 61±0.9° in walking), perhaps indicating a limit to the degree of elbow flexion possible. In contrast, maximum elbow extension is significantly greater during retraction in swimming, potentially facilitating aquatic thrust production (Pace et al., 2001). It is also possible that the restricted range of elbow extension during terrestrial locomotion would help to minimize vertical fluctuations of the center of mass, potentially minimizing energy loss during walking. A more terrestrial emydid, the ornate box turtle (*Terrapene ornata*), has recently been identified as an economical walker (Zani and Kram, 2008), though contributing limb kinematic mechanisms have not been addressed.

Foot kinematics also differ significantly between water and land. In swimming, foot movements lead to a feathered orientation for much of humeral protraction (recovery phase), helping to minimize drag as the foot is drawn forwards through the water (Fig. 2.3D). During walking, however, such a feathered forefoot orientation is not maintained during humeral protraction, perhaps in part because drag is not a substantial factor during swing phase on land.

Effect of habitat on forelimb muscle activation patterns

The majority of the pectoral girdle muscles examined are active at the portions of the limb cycle predicted based on their anatomical positions. Coracobrachialis, pectoralis, and supracoracoideus (both heads) were confirmed to be active during humeral retraction and depression, whereas latissimus dorsi and deltoid were confirmed to be active during humeral protraction and elevation (Fig. 2.4). Triceps, a predicted elbow extensor, was likewise found to be active during elbow extension.

However, the EMG data yielded some surprising findings. For example, with regard to burst intensity, I had predicted that limb protractors might show higher mean amplitude bursts during swimming to overcome the greater resistance to movement through water versus air, whereas limb retractors might show greater activity on land in order to support the body without the benefit of buoyancy. Instead, most muscles did not exhibit significant differences in mean burst amplitude between habitats, and the few that did, including coracobrachialis, subscapularis (lat approach), and the anterior head of supracoracoideus, ran contrary to my predictions, with all of these retractors exhibiting significantly higher mean amplitudes during swimming (Table 2.2).

Differences in the timing of activity patterns between habitats were more common than differences in burst intensity. Some of these seem to be straightforward reflections of differences in the durations of limb cycle phases between swimming and walking. For example, the later onset of coracobrachialis in water likely reflects the later initiation of humeral retraction during swimming, while the earlier offset of latissimus dorsi on land matches the earlier end of protraction during walking (Fig. 2.4). However, some differences in the timing of muscle activity between habitats are more surprising. For instance, while pectoralis is confirmed to be active during retraction in both habitats, swimming *T. scripta* display an additional early burst of activity that occurs during protraction (Fig. 2.4). This early burst in swimming is not present in all swimming cycles, but may act to stabilize the shoulder during humeral protraction when the limb is being moved through the dense aquatic medium. The lack of this stabilizing burst during walking may relate to the different demands being placed on the limbs during locomotion in water versus air. The ventrally situated pectoralis is in an anatomical position to depress the forelimb when it contracts. The timing of the early stabilizing activity seen during swimming would, during walking, occur during swing phase. During swing phase the forelimb is quite literally “swung” forward and upward, with walking characterized by much greater humeral elevation than swimming (Fig. 2.3B, Table 2.1). In addition to the shoulder likely not requiring much stabilization while moving through less resistant air versus water, additional pectoralis activity during terrestrial swing phase would not only act counter to the forward movement of the limb but also counter to its elevation required to clear the ground.

Another unexpected finding, and difference in pattern between swimming and walking, is in the activity of subscapularis. While the posterior “cor approach” shows a single burst of activity for both habitats, the more anterior “lat approach” shows two bursts during walking and only a single burst during swimming (Fig. 2.4). In addition, while this muscle is predicted to act during humeral elevation based on anatomical position (Walker, 1973), most of its activity occurs during humeral retraction and depression. Walking *T. scripta* exhibit significantly greater humeral elevation, which may account for the early burst from the anterior (“lat approach”) regions of subscapularis on land. Although the sample size for this muscle is limited (N=2 for “cor approach”, N=1 for “lat approach”), this muscle may be acting as a brake to reduce the amount of humeral depression during the thrust-producing power stroke.

Triceps also shows patterns that were not initially predicted. Triceps shows two bursts of activity in walking and swimming; while the early burst is always present in swimming, the later burst was variable, and both bursts were always present in walking. During walking, two periods of elbow extension occur roughly coincident with the two bursts of triceps activity (Figs 3C, 4). During swimming, however, elbow extension only occurs from approximately 20-70% of the limb cycle, coinciding with the early burst of triceps activity. The later triceps activity during swimming may act to stabilize the elbow as the limb is brought closer to the body during thrust phase. Thus, identification of kinematic differences between environments was insufficient to predict the full range of differences in the motor patterns of the slider forelimb between water and land.

Comparison of forelimb and hindlimb motor patterns

Functional requirements for moving through an aquatic environment are quite different from those for moving on land. Predictions for the modulation of limb muscle motor patterns between these different habitats suggest that limb protractors might show more intense activity during swimming than in walking in order to accommodate the greater viscosity of water compared to air, while limb extensors might show more intense activity on land because bearing weight while moving could require higher forces than aquatic propulsion. However, these predictions are not universally borne out for the forelimb muscles I examined. Data for *T. scripta* show no significant differences in intensity between swimming and walking for protractors. In fact, in most cases amplitude is very similar between swimming and walking for the two main forelimb protractors, latissimus dorsi and deltoid. Though not matching expectations based on physical differences between environments, EMG modulations for *T.*

scripta forelimb protractors also differ from those seen in functionally analogous hindlimb protractors. The femoral protractors iliofemoralis (ILF) and puboischiofemoralis internus (PIFI), showed similar burst timing between swimming and walking in *T. scripta*, but different patterns of intensity modulation, with ILF showing greater amplitude in swimming as expected, but PIFI showing greater amplitude in walking (Gillis and Blob, 2001; Blob et al., 2008).

Modulation patterns exhibited by forelimb retractors and extensors also differed from predictions based on physical differences between the environments, as I found no differences in amplitude between swimming and walking for triceps or pectoralis, and coracobrachialis, subscapularis, and supracoracoideus exhibited higher amplitude bursts during swimming rather than walking. However, while counter to expectations based on physical differences between environments, patterns for the latter forelimb muscles do match patterns observed for functionally analogous hindlimb retractors femorotibialis (FT) and flexor tibialis internus (FTI) in *T. scripta* (Gillis and Blob, 2001; Blob et al., 2008), which also showed greater amplitude bursts during swimming. At least for propulsive phase muscles, motor pattern modulations between water and land in *T. scripta* are largely parallel between the fore- and hindlimb. It is possible that despite support of the body by buoyancy, the intensity of muscular effort required for propulsive rowing strokes through a viscous aquatic medium is greater than has previously been appreciated, perhaps because force transmission may be less efficient in water versus on land. As a result, it might be reasonable to expect propulsive phase muscles (retractors) to show increased activity during swimming. Increased EMG amplitude does not necessarily correlate with higher force, because the force exerted by a muscle is dependent on both velocity and length (Loeb and Gans, 1986; Lieber, 2002), and differences in kinematics between environments could contribute to changes in both parameters. However, the potential for higher muscular forces during swimming might elevate expectations for the loads that would be placed on the limb skeleton during aquatic locomotion (Butcher and Blob, 2008; Butcher et al., 2008), though the direction of bone loading may differ substantially between the two habitats.

Comparisons to environmental modulations of motor patterns in other taxa

In most species examined to date, locomotion in different environments seems to consistently be accompanied by alterations in activity of major locomotor muscles (Ashley-Ross and Lauder, 1997; Gillis, 1998a; Gillis, 1998b; Gillis, 2000; Gillis and Biewener, 2000; Gillis and Biewener, 2001; Gillis and Blob, 2001; Higham and Jayne, 2004; Blob et al., 2008). These differences, which may be in the form of intensity, duration, timing, or some combination of these variables, can even change the functional role of muscles between environments (Gillis and Blob, 2001). However, differences in the timing of muscle activity more commonly correlate with kinematic differences between habitats, and while changes in EMG amplitude between land and water are widespread, predicted differences based on the differing functional requirements of these environments are not always seen (Gillis and Blob, 2001; Blob et al., 2008).

A broad question that has received attention in many studies is which components of functional systems change during the evolution of new functions or behaviors (Westneat and Wainwright, 1989; Reilly and Lauder, 1992; Lauder and Reilly, 1996). The idea that new patterns of movement can be achieved while conserving the patterns of muscle activity is commonly described as the neuromotor conservation hypothesis (Peters and Goslow, 1983; Smith, 1994). Despite the drastic diversity in structure and locomotion across vertebrate taxa, remarkably similar patterns of limb muscle activation have been documented across behaviors ranging from sprawling and upright terrestrial locomotion to flight (Peters and Goslow, 1983; Goslow et al., 1989; Dial et al., 1991; Fish, 1996; Goslow et al., 2000). This has led to the hypothesis that patterns of neuromotor control for homologous tetrapod muscles are evolutionarily conserved, despite modifications to the limb muscles and skeleton for different uses (Jenkins and Goslow, 1983; Peters and Goslow, 1983; Smith, 1994).

While *T. scripta* definitely exhibit some differences in muscle activity between swimming and walking (timing, intensity, and number of bursts), the basic motor patterns between these behaviors are, in many ways, more similar than might be expected based on the dramatically different environmental conditions in which they are used. The differences that do exist typically correlate well with the required differences in kinematics between water and air.

Examination of additional species could test if such patterns hold more broadly across turtles between environments. Additionally, with the presence of two distinct patterns of forelimb motion in lineages of swimming turtles — dorsoventral flapping in sea turtles (Davenport et al., 1984; Wyneken, 1997) versus the anteroposterior rowing typical of most aquatic turtle species (Pace et al., 2001), evaluation of the conservation of swimming motor patterns across turtle species could provide a fruitful test of how muscle actions may evolve in concert with novel functions.

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CHAPTER THREE

FORELIMB KINEMATICS AND MOTOR PATTERNS OF SWIMMING LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*): ARE MOTOR PATTERNS CONSERVED IN THE EVOLUTION OF NEW LOCOMOTOR STRATEGIES

Abstract

Novel locomotor functions in animals may evolve through changes in morphology, muscle activity, or a combination of both. The idea that new functions or behaviors can arise solely through changes in structure, without concurrent changes in the patterns of muscle activity that control movement of those structures, has been formalized as the ‘neuromotor conservation hypothesis’. In vertebrate locomotor systems, evidence for neuromotor conservation is found across transitions in terrestrial species and into fliers, but transitions in aquatic species have received little comparable study to determine if changes in morphology and muscle function were coordinated through the evolution of new locomotor behaviors. To evaluate the potential for neuromotor conservation in an ancient aquatic system, I quantified forelimb kinematics and muscle activity during swimming in the loggerhead sea turtle (*Caretta caretta*). Loggerhead forelimbs are hypertrophied into wing-like flippers that produce thrust via dorsoventral forelimb flapping. I compared kinematic and motor patterns from loggerheads to previous data from the slider (*Trachemys scripta*), a generalized freshwater species exhibiting unspecialized forelimb morphology and anteroposterior rowing motions during swimming. For some forelimb muscles,

comparisons between *Caretta* and *Trachemys* support neuromotor conservation: for example, coracobrachialis and latissimus dorsi show similar activation patterns. However, other muscles (deltoideus, pectoralis, triceps) do not show neuromotor conservation, with deltoideus changing dramatically from a limb protractor/elevator in sliders to a joint stabilizer in loggerheads. Thus, during the evolution of flapping in sea turtles, drastic restructuring of the forelimb was accompanied by both conservation and evolutionary novelty in limb motor patterns.

Introduction

A major focus of evolutionary studies of musculoskeletal function, particularly of vertebrate feeding and locomotion, is understanding how changes in anatomical structures are correlated with changes in muscle activity patterns during evolutionary changes in function or behavior. Despite dramatic variations in structure and function across vertebrate taxa, remarkably similar patterns of muscle activation have been documented across taxa that span diverse ranges of behavior in both feeding and locomotor systems (Peters and Goslow, 1983; Goslow et al., 1989; Westneat and Wainwright, 1989; Dial et al., 1991; Fish, 1996; Goslow et al., 2000). Such studies led to the hypothesis that patterns of neuromotor control often are conserved evolutionarily across behavioral transitions, even when morphological changes are dramatic (e.g., legs to wings: Jenkins and Goslow, 1983; Dial et al., 1991). The hypothesis that new movement patterns can be achieved while conserving patterns of muscle activity is known as the ‘neuromotor conservation hypothesis’ (see Smith, 1994 for review). Although a number of its invocations have been criticized (Smith, 1994), it inspired numerous studies seeking to explain and understand the evolutionary diversity of functional performance (Jenkins and Weijs, 1979; Jenkins and Goslow, 1983; Peters and Goslow, 1983; Goslow et al., 1989; Dial et al., 1991; Reilly and Lauder, 1992; Lauder and Reilly, 1996; Goslow et al., 2000).

Initial studies of neuromotor conservation in tetrapod locomotion focused on terrestrial limb use and on transitions to flight (Jenkins and Weijs, 1979; Jenkins and Goslow, 1983; Peters and Goslow, 1983; Goslow et al., 1989; Dial et al., 1991; Goslow et al., 2000). However, dramatic structural changes also can be found through the evolution of locomotion in lineages of aquatic tetrapods (Fish, 1996), and whether neuromotor firing patterns were conserved through such transitions is unknown.

Among tetrapod lineages that frequently use aquatic locomotion, turtles provide strong advantages for studies of neuromotor conservation during locomotor evolution. Because all turtles have a rigid shell comprised of fused vertebrae, ribs and dermal elements, movement of the body axis is precluded, meaning that propulsive forces are generated almost exclusively by the limbs (Zug, 1971; Wyneken, 1997; Blob et al., 2008). Thus, evaluations of differences in limb motor patterns across taxa should not be confounded significantly by the contributions of other structures to propulsion, like flexible bodies, tails, or specialized fins (Blake et al., 1995; Walker, 2000; Fish, 2002; Fish and Nicastro, 2003; Rivera et al., 2006). Turtles display a diverse range of locomotor styles and associated limb morphology. All but one clade, the tortoises, are primarily aquatic (Ernst and Lovich, 2009; Gosnell et al., 2009). While there are many differences among species with regard to their locomotion in aquatic habitats (Pace et al., 2001; Blob et al., 2008; Renous et al., 2008), one of the most striking examples is the difference between the two basic types of swimming found in turtles – rowing and flapping. Rowing is the more common and ancestral form of swimming in turtles and is used by all but one freshwater species. Rowing is characterized by anteroposterior (i.e., front-to-back) movements of the limbs in which the forelimb of one side moves essentially in phase with the contralateral hindlimb, so that forelimbs (and hind limbs) of opposite sides move asynchronously (Pace et al., 2001; Rivera et al., 2006). In contrast, flapping (also referred to as aquatic flight) is characterized by synchronous, largely dorsoventral (i.e., up-and-down) movements of the forelimbs, and is thought to produce thrust on both upstroke and downstroke (Walker, 1971; Walker, 1973; Davenport et al., 1984; Renous and Bels, 1993; Walker and Westneat, 2000). All seven species of sea turtles and one species of freshwater turtle, *Carettochelys insculpta*, employ this mode of swimming, which is facilitated by derived modification of the forelimbs into elongate, semi-rigid flippers.

Novel behaviors, including patterns of limb motion such as aquatic flapping, might arise through modification of structures, modification of patterns of muscle activation, or some combination of both. Despite the dramatic differences in external morphology and humerus shape between the forelimbs of rowing and flapping turtles, all turtles share the same basic limb musculature [i.e., no major muscles were lost or added in the evolution of aquatic flight (Walker, 1973)]. This means that rowers and flappers with disparate limb morphology must execute their different styles of swimming either strictly as a mechanical consequence of those morphological differences (i.e., without changes in the underlying motor patterns), or through a combination of differences in morphology as well as motor patterns. The latter would indicate a lack of conservation, while the former would provide support for the hypothesis of neuromotor conservation in the evolution of aquatic flight. The extent to which divergent motor patterns contribute to the diversity in locomotor behaviors used by swimming turtles has not been evaluated (Blob et al., 2008). Comparisons of forelimb motor patterns across taxa that swim via rowing versus flapping would, therefore, allow evaluations of how divergence in limb neuromotor control contributes to divergence of limb kinematics and locomotor behavior through evolution in this lineage.

In this study, I tested whether differences in muscle function correlated with changes in limb morphology and locomotor behavior in turtles. I quantified forelimb motor patterns exhibited during flapping-style swimming by *Caretta*

caretta (the loggerhead sea turtle; Linnaeus, 1758), and compared these motor patterns to those recently published for the rowing-style swimming of a generalized freshwater turtle, the red-eared slider *Trachemys scripta* Schoepf, 1792 (see Chapter 2). *Trachemys scripta* is a member of the emydid lineage and, as such, is not a member of the sister clade to sea turtles. They are generally similar to the majority of freshwater turtles in their limb morphology and swimming style, making them a reasonable model to represent the basal condition for turtle swimming and compare with *C. caretta*. Moreover, the sister taxa to sea turtles [the kinosternids and chelydrids (Barley et al., 2010)] typically walk along the bottom of aquatic habitats rather than swim (Zug, 1971), making measurement of comparable variables difficult. This test of the neuromotor conservation hypothesis helps to clarify the mechanisms by which new locomotor strategies evolve.

Materials and Methods

Experimental animals

Data were collected from four juvenile loggerhead sea turtles (*C. caretta*) that were similar in carapace length (59-65 mm; mean ± S. E. M. = 62±1.0 mm) and body mass (31.7-45.8 grams; 41.4±1.3 grams). Hatchlings were collected from nesting beaches in Florida and were captive reared at the Florida Atlantic University Gumbo Limbo Laboratory for a separate unrelated study; all were later released into the wild. Turtles were housed individually (to minimize aggression and avoid competition for food) in 20 cm X 20 cm X 20 cm plastic mesh baskets, which were placed into large tanks equipped with flow-through filtered seawater maintained at 27°±2 C (the approximate thermal conditions in the Gulfstream). A 12 h light: 12 h dark photocycle was maintained with natural spectrum fluorescent lighting. Turtles were fed once daily using an in-house manufactured diet (detailed in Stokes et al., 2006; for further details on housing conditions and diet, see Dougherty et al., 2010). Studies were conducted at Florida Atlantic University in accordance with IACUC guidelines (protocol 07-17 and Marine Turtle Permits #TP073 and TE056217-2). Experimental procedures followed those of the previous study of slider turtles (see Chapter 2) as closely as possible to facilitate comparisons of data between these species.

Collection and analysis of kinematic data

Locomotor trials (see Appendix D) were conducted in a custom-built aquarium with transparent glass sides and bottom (LxWxH = 76 x 32 x 30 cm; ~1200 L). Kinematic data were collected simultaneously in lateral and ventral views (100 Hz) using two digitally synchronized high-speed video cameras (Phantom V4.1, Vision Research, Inc.; Wayne, NJ, USA). Ventral views were obtained by directing the ventral camera at a mirror oriented at a 45° angle to the transparent bottom of the tank. Turtles were filmed swimming in still water at 27º±2 C. Synchronized video (for kinematic analysis) and EMGs (for motor patterns) were collected from each turtle, yielding 8-14 limb cycles per turtle.

From collected video footage, complete three-dimensional kinematic data could be synchronized with EMGs for three of the four loggerhead turtles (see Appendix D); these data were supplemented with EMG data for latissimus dorsi for a fourth individual. I synchronized those EMG data based on the start of humeral elevation and completion of humeral depression (see Appendix E).

To facilitate digitization of animal movement from videos, nontoxic white dots provided high-contrast points on the following 14 anatomical landmarks (Fig. 3.1): anterior tip of the nose; shoulder; elbow; digits 1, 3, and 5 on the foreflipper; two landmarks on the carapace; and an anterior, posterior, right, and left point on the plastron (Fig. 3.1). Landmark positions were digitized frame-by-frame in each video using QuickImage (Walker, 1998) or DLTdataViewer2 (Hedrick, 2008). The three-dimensional coordinate data generated were then processed using custom Matlab (Student Ver. 7.1, MathWorks, Inc.; Natick, MA, USA) routines to calculate limb kinematics during swimming; calculations include protraction and retraction angles of the humerus, elevation and depression angles of the humerus, and extension and flexion angles of the elbow. Calculated kinematic values from each limb cycle were fit to a quintic spline using QuickSAND (Walker, 1998) to smooth the data, and interpolated to 100 values in order to normalize all limb cycles to the same duration. This transformation allowed me to compare locomotor cycles of different absolute durations and calculate average kinematic profiles and standard errors for each variable through the course of swimming trials.

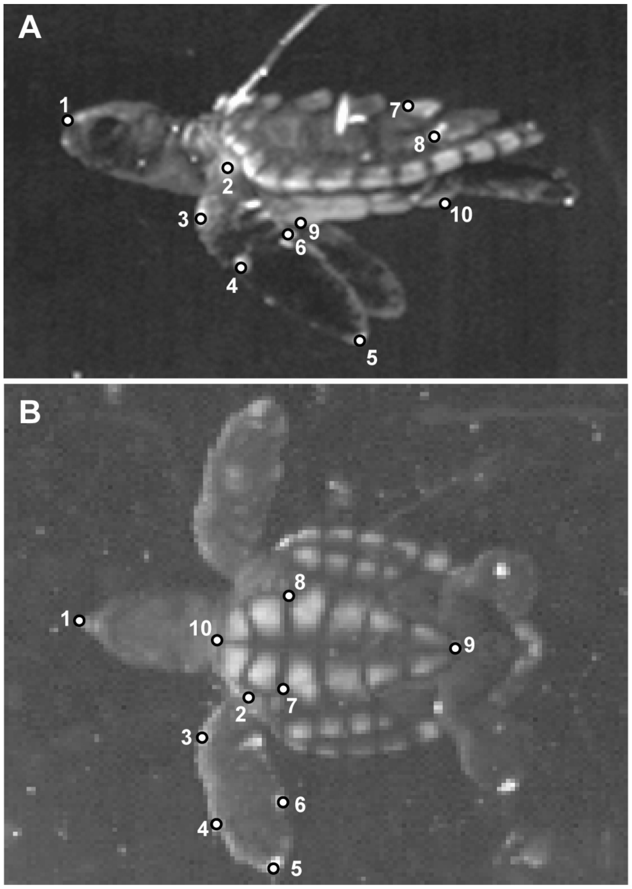

Fig. 3.1. Representative still images from lateral (A) and ventral (B) videos showing landmarks digitized for kinematic analysis of *Caretta caretta*. Landmarks common to both views include: 1- tip of the nose, 2- shoulder, 3- elbow, 4- digit 1, 5- digit 3 (tip of flipper), and 6- digit 5. Additional lateral landmarks (A) include: 7- high landmark on carapace and 8- low landmark on carapace. Additional ventral landmarks (B) include: 7- point on left side of plastron, 8- point on right side of plastron, 9- posterior point on plastron, and 10- anterior point on plastron.

Standard conventions for limb angle definitions from the previous work (see Chapter 2) were applied. Briefly, a humeral protraction/retraction angle of 0° indicates that the humerus is perpendicular to the midline of the turtle; while an angle of 90° indicates a fully protracted forelimb with the distal end of the humerus directed anteriorly (an angle of -90° would indicate a fully retracted forelimb with the distal tip of the humerus directed posteriorly). A humeral elevation/depression angle of 0° indicates that the humerus is in the turtle's frontal plane through the shoulder (i.e., horizontal plane in relation to the tank), with angles greater than zero indicating elevation above the long axis (distal end above proximal end) and negative angles indicating depression of the humerus (distal end lower than proximal end). Extension of the elbow is indicated by larger extension/flexion angles and flexion is indicated by smaller values: an elbow angle of 0° (while not anatomically possible) would indicate a fully flexed elbow (i.e., humerus perfectly parallel to radius and ulna), while 180° would indicate a fully extended elbow. Flipper (i.e., forefoot in *T. scripta*) orientation angle was also calculated as the angle between a vector pointing forwards along the anteroposterior midline (also the path of travel) and a vector emerging from the palmar surface of a plane defined by the tips of digits 1 and 5 and the elbow; this angle was transformed by subtracting 90° from each value (Pace et al., 2001). A high-drag orientation of the flipper blade (or forefoot paddle) with the palmar surface directed opposite to the direction of travel (and in the same direction as the flow of water) is indicated by an angle of 90°, and a perfect low-drag orientation of the flipper blade is indicated by an angle of 0°.

Collection and analysis of electromyographic data

Concurrent with video acquisition, electromyography (EMG) was used to measure muscle firing patterns of target forelimb muscles (Loeb and Gans, 1986). Following previously established protocols (Loeb and Gans, 1986; Westneat and Walker, 1997; Gillis and Blob, 2001; Blob et al., 2008; Schoenfuss et al., 2010; see Chapter 2), 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 forelimb using hypodermic needles. Local anesthesia at the implant sites was provided with lidocaine infusion prior to procedures. External landmarks for implants were determined prior to data collection through dissection of preserved specimens, helping to ensure accurate placement of electrodes. Due to the protected status of loggerhead sea turtles, I was not permitted to follow experiments with verification dissections that would have required sacrifice of study animals. Instead, implants were practiced on preserved specimens using external implant landmarks as a guide; once implants were used to successfully implant target muscles five times in a row with no errors, the landmarks were considered valid. Implants were done in live animals only after achieving competency implanting electrodes in target muscles.

Up to 10 implants were performed for each experiment, with target muscles receiving multiple electrodes (2-3) to help ensure successful recordings even if some electrodes failed. Electrode wires exiting the forelimb were allowed several centimeters of slack before being bundled and glued together into a cable that was directed dorsally and sutured to the skin just anterior to the carapace. During locomotor trials, EMG signals were relayed from the electrodes in each turtle to a Grass 15LT amplifier system (West Warwick, RI, USA) for amplification (10,000 times) and filtering (60Hz notch filter, 30Hz-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. Kinematic data were synchronized with electromyographic data by triggering a signal generator that simultaneously produced a light pulse visible in the video and a square wave in the EMG data. EMG data were analyzed using custom LabVIEW software routines to identify bursts of muscle activity (Schoenfuss et al., 2010; see Chapter 2).

I focused on five target muscles (Fig. 3.2) for this study, covering all major planes of motion of the forelimb during swimming. Predicted actions for each muscle were based on anatomical position (Walker, 1973; Wyneken, 2001). The coracobrachialis is positioned posterior to the humerus and expected to retract the forelimb. The pectoralis is a large, triangular sheet that extends widely from approximately the plastral midline to converge and insert on the flexor border of the lateral process of the humerus, and is predicted to retract and depress the humerus. The latissimus dorsi is positioned anterior and dorsal to the humerus along the scapula and is predicted to protract and elevate the limb. The deltoideus is located more ventrally, attaching to the plastron close to its

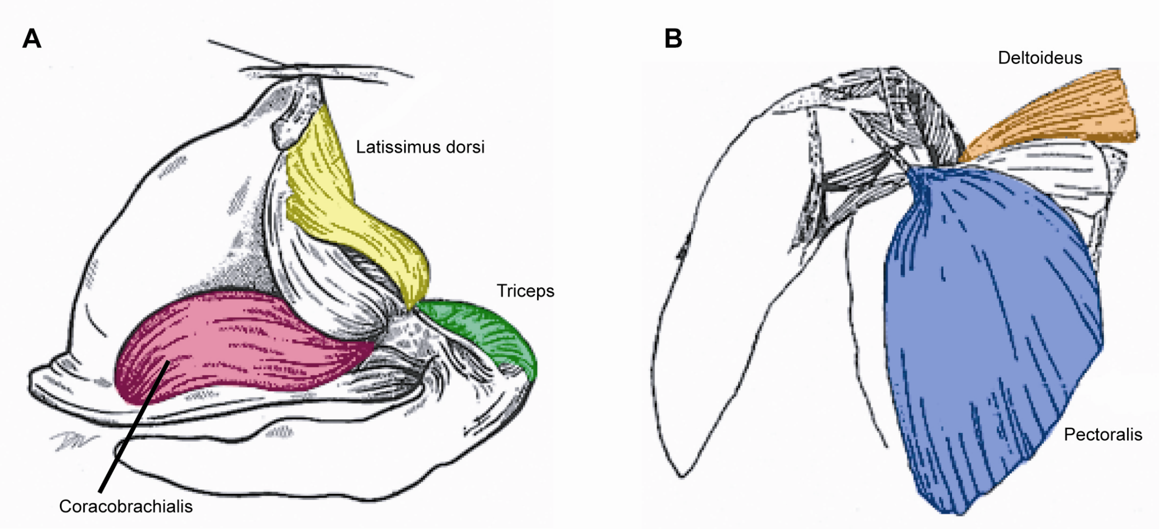

Fig. 3.2. Illustration showing five target muscles from which electromyographic data were collected. (A) Lateral view of the right forelimb musculature of *C. caretta*; modified from Wyneken (2001). (B) Ventral view of the left forelimb musculature of *C. caretta*; modified from Wyneken (1997). Predicted muscle actions are based on their anatomical positions (Walker, 1973; Wyneken, 2001). Coracobrachialis (pink) is situated posterior to the humerus and expected to retract the forelimb. The most ventral target muscle, pectoralis (blue) extends from the plastral midline towards the anterior margin of the bridge of the shell to a tendon that inserts on the lateral process of the humerus, and is predicted to retract and depress the humerus. Latissimus dorsi (yellow) is anterior and dorsal to the humerus and runs from the anterolateral scapula and dorsal carapace to the proximal humerus; it is predicted to protract and elevate the forelimb. More ventrally and cranially is the deltoideus (orange), which runs from the plastron to the proximal humerus near the shoulder joint and is predicted to protract and elevate the humerus. Triceps complex (green) is located on the extensor surface of the arm, running from the shoulder joint distally to the elbow, and is predicted to act in flipper blade extension at the elbow.

midline and running to the shoulder joint, but also with predicted actions of humerus protraction and elevation. Finally, the triceps complex is located on the extensor surface of the arm, running from the shoulder joint to the elbow, and is predicted to act in elbow extension.

Statistical analyses

To assess general patterns of movement and muscle function, the overall mean and standard error of each variable was calculated for all swimming trials. Muscle activity variables include, for each muscle: (i) onset, (ii) offset, and (iii) duration. Kinematic variables include: (i) maximum protraction, retraction, elevation, and depression of the humerus, (ii) maximum elbow extension and flexion, (iii) anteroposterior and dorsoventral excursion of the humerus, (iv) elbow excursion, (v) percentage of the cycle at which maximum elbow extension occurs, (vi) the percentage of the limb cycle at which a switch from elevation to depression occurs, (vii) the percentage of the limb cycle at which a switch from protraction to retraction occurs, and (viii) the maximum, minimum, and range of feathering of the forefoot. Because the maximum values for each limb cycle do not always occur at the same percentage of the limb cycle, it is possible that the average of the maximum values calculated for all limb cycles may be masked (appear lower) in average kinematic profiles. I compare data for loggerheads (hereafter *Caretta*) to that previously published for rowing-style swimming in sliders (hereafter *Trachemys*) (see Chapter 2) to assess the differences in kinematics between a flapping species and a generalized rowing species, and to assess whether motor patterns during swimming are similar or different between the species. I used Systat (v.12) for all statistical analyses, and $P<0.05$ as the criterion for significance.

To determine whether swimming forelimb kinematics and motor patterns differ between *Caretta* and *Trachemys* (presented in Chapter 2), I conducted two-way mixed-model nested analyses of variance (ANOVA), with species as a fixed factor and individual (nested within species) as a random factor. Two-way, mixed model, nested ANOVAs (corrected for unbalanced sampling) were performed separately on each variable, with one set performed on data from each kinematic variable (Table 3.1) and one on each muscle timing variable (Table 3.2). In tabular data summaries I provide d.f. and *F*-values to clarify the potential effects of making multiple comparisons.

Results

Timing of muscle activity relative to limb motion was measured for 8-14 swimming trials from each of the four sea turtles, with three-dimensional kinematics calculated from three of the four animals (see Appendix D). The number of trials from which EMG data were collected varied across individuals and muscles due to differences in the success of electrode implants. Plots depicting the general pattern of muscle activation during swimming were constructed using all collected EMG data for *Caretta* and published data for *Trachemys* (see Chapter 2) (see Appendix E). A summary of sample sizes from each individual, by species, is given for statistical analyses (see Appendices D, E).

Table 3.1. Mean values and standard errors of humeral kinematic variables and F-values for the main effect of species from two-way mixed model nested ANOVAs performed separately on each variable

Variable	*Caretta caretta*	*Trachemys scripta*	*F*-value (d.f. 1,8)
Maximum humeral depression[1]	-51±2.6	-8±0.6	171.34***
Maximum humeral elevation[1]	10±3.7	20±0.7	3.19
% of limb cycle at maximum elevation[2]	51±2.5	43±1.0	5.09*
Dorsoventral humeral excursion angle[3]	61±4.5	28±0.7	36.12***
Maximum humeral retraction[1]	26±2.0	8±0.8	16.27**
Maximum humeral protraction[1]	64±2.2	115±1.4	48.22***
% of limb cycle at maximum protraction[2]	44±2.9	43±0.6	0.42
Anteroposterior humeral excursion angle[3]	38±2.4	107±1.7	48.50***
Maximum elbow flexion[1]	93±3.6	61±1.3	6.69*
Maximum elbow extension[1]	139±3.1	123±0.9	8.43*
% of limb cycle at maximum elbow extension[2]	59±4.0	68±1.3	3.76
Elbow excursion angle[3]	46±3.3	62±1.5	1.95
Maximum forefoot feathering[1]	54±3.1	78±1.1	21.63***
Minimum forefoot feathering[1]	-18±3.0	-5±1.2	4.76
Total Forefoot feathering excursion[3]	72±2.7	83±1.2	3.41

[1] Values are angles in degrees

[2] Values represent a percentage of the limb cycle

[3] Values represent the total angular excursion

**P*≤0.05; ***P*≤0.01; ****P*≤0.001

Table 3.2. Mean values and standard errors for EMG timing variables and F-values for the main effect of species

Variable	*Caretta caretta*	*Trachemys scripta*	F-value	d.f.
Coracobrachialis				
Onset	62±1.3	51±1.0	4.04	1,4
Offset	84±1.3	85±0.5	0.95	1,4
Duration	21±1.4	34.9±1.2	4.10	1,4
Pectoralis Burst #1[1]				
Onset	57±1.7	4±0.7	122.81***	1,3
Offset	78±1.1	24±1.3	93.61**	1,3
Duration	21±1.3	20±1.7	0.27	1,3
Pectoralis Burst #2[1]				
Onset	------------	62±1.5	0.23	1,5
Offset	------------	89±0.7	6.93*	1,5
Duration	------------	28±1.3	0.51	1,5
Latissimus dorsi[2]				
Onset	91±0.9	83±1.0	2.76	1,5
Offset	39±1.2	35±0.9	1.53	1,5
"Burst #1" Duration	37±1.2	35±0.9	0.57	1,5
"Burst #2" Duration	8±0.9	16±1.0	3.04	1,5
Total Duration	44±1.6	51±1.3	1.32	1,5
Deltoideus[3]				
Onset	60±1.3	96±0.4	1182.10***	1,7
Offset	84±1.0 [-16±1.0]	33±0.9	89.16***	1,7
Total Duration	24±1.4	32±1.7	0.69	1,7
Triceps (versus Burst #1 in *T. scripta*)[4]				
Onset	90±0.8	23±1.3 [123±1.3]	8.86*	1,5
Offset	44±1.5	51±1.5	6.05	1,6
Total Duration	45±1.8	28±0.9	10.27*	1,6

Table 3.2., continued

Variable	*Caretta caretta*	*Trachemys scripta*	F-value	d.f.
Triceps (versus Burst #2 in *T. scripta*)[4]				
Onset	90±0.8	83±1.0	1.70	1,5
Offset	44±1.5	91±0.6 [-9±0.6]	249.52***	1,6
Total Duration	45±1.8	8±0.5	76.45***	1,6

Two-way mixed model nested ANOVAs performed separately on each variable.

[1] *C. caretta* exhibits one discrete burst of pectoralis activity, whereas *T. scripta* shows two bursts of activity (with the early burst being variable). The single burst in *C. caretta* (Burst #1) was separately compared to both Burst #1 and Burst #2 in *T. scripta.*

[2] Latissimus dorsi presents as a continuous burst of activity that spans the depression to elevation (and retraction to protraction) phase shift. Quotation marks (i.e., "Burst #1" and "Burst #2") are used to indicate the early and late activity, respectively, of such a muscle. Onset is the start of “Burst #2” and offset is the end of “Burst #1”.

[3] *C. caretta* exhibits one discrete burst of deltoideus activity, whereas *T. scripta* shows one continuous burst of activity that spans the switch from retraction to protraction. For *C. caretta*, statistical analysis of offset uses [transposed value], by subtracting 100. For *T. scripta*, onset is the start of “Burst #1” and offset is the end of “Burst #2”.

[4] *C. caretta* exhibits one long continuous burst of triceps activity that spans the switch from depression to elevation. For *C. caretta*, onset is the start of “Burst #2” and offset is the end of “Burst #1”. Triceps activity in *C. caretta* is compared to both discrete bursts of activity observed in *T. scripta*. For *T. scripta*, statistical comparison of onset of Burst #1 uses [transposed value], by adding 100; comparison of offset of Burst #2 uses [transposed value], by subtracting 100.

Total duration is the combined early and late durations, though not all trials showed both (see Appendix D).

Values are means ± standard error

**P*≤0.05; ***P*≤0.01; ****P*≤0.001

Kinematics of swimming: flapping versus rowing

Limb cycles for each species were defined based on the major plane of motion of the forelimb. Therefore, a flapping limb cycle in *Caretta* was defined as starting at the beginning of humeral elevation, lasting through humeral depression, and ending at the start of the next cycle of elevation. This definition differs for rowing in *Trachemys*, in which the limb cycle was defined as humeral protraction followed by retraction. While the limb cycle was defined differently for *Caretta* and *Trachemys*, it should be noted that humeral elevation and protraction are essentially concurrent in both species, as are humeral depression and retraction.

In general, forelimb movement during swimming in *Caretta* is characterized by humeral elevation, and to a lesser degree protraction, that both reach a single peak before being followed by extensive humeral depression accompanied by a small degree of retraction (Fig. 3.3A, B). The elbow of *Caretta* is extended through humeral elevation, and reaches a single peak shortly after the start of humeral depression. As the humerus is depressed, the elbow is flexed.

The single peak of humeral elevation in *Caretta* occurs at 52±2.5% (mean ± S. E. M.) of the limb cycle, which is significantly later than that observed in *Trachemys* at 42±1.0% of the limb cycle (Fig. 3.3A, Table 3.1). While the range of dorsoventral humeral motion is far greater in *Caretta* (61±4.5° versus 28±0.7°; Fig. 3.3A, Table 3.1), this is achieved primarily through a vastly greater degree of

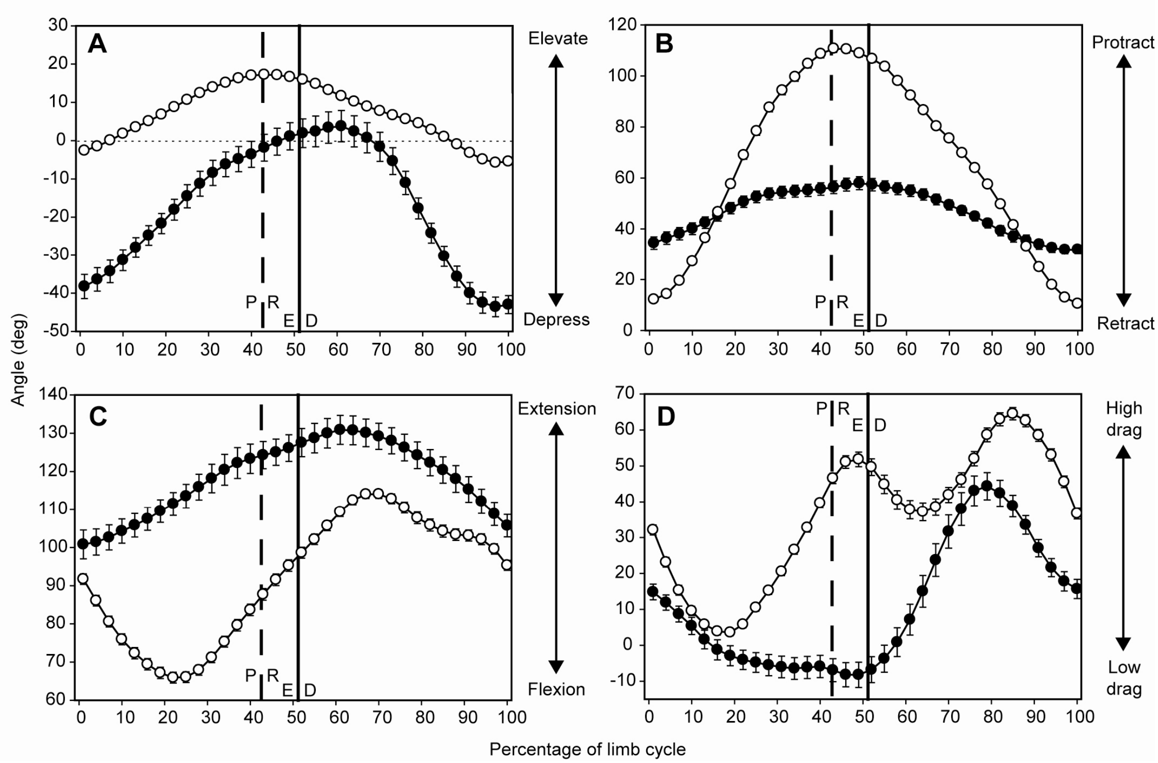

Fig. 3.3. Mean kinematic profiles for *C. caretta* (filled symbols) and *T. scripta* (open symbols) during swimming. Data for *T. scripta* from Chapter 2. Each trial from *C. caretta* was normalized to the same duration and angle values interpolated to 100 points representing the complete duration of the limb cycle. The limb cycle for *C. caretta* is defined as elevation of the humerus followed by depression; a limb cycle for *T. scripta* is defined as protraction of the humerus followed by retraction. Mean angle values ± S.E.M. are plotted for every third increment (every 3% through the cycle) for all individuals. Solid vertical lines demarcate the switch from elevation (E) to depression (D) in *C. caretta*; dashed vertical lines demarcate the switch from protraction (P) to retraction (R) in *T. scripta*. (A) Humeral elevation and depression (i.e., angle from the horizontal plane). An angle of 0° indicates that the humerus is in the horizontal plane. Angles greater than zero indicate elevation above the horizontal (distal end above proximal end) and negative angles indicate depression of the humerus (distal end lower than proximal end). (B) Humeral protraction and retraction (i.e., angle from the transverse plane). An angle of 0° indicates that the humerus is perpendicular to the midline of the turtle, while an angle of 90° indicates a fully protracted forelimb with the distal end of the humerus directed anteriorly (an angle of -90° would indicate a fully retracted forelimb with the distal tip of the humerus directed posteriorly). (C) Elbow flexion and extension. Extension is indicated by larger angles and flexion is indicated by smaller angles. An angle of 0° indicates complete flexion, while 180° indicates a fully extended elbow. (D)

Forefoot orientation angle is calculated as the angle between a vector pointing forwards along the anteroposterior midline (also the path of travel) and a vector emerging from the palmar surface of a plane defined by the tips of digits 1 and 5 and the elbow; this angle is transformed by subtracting 90° from each value. Data originally reported for *T. scripta* forefoot orientation in Chapter 2 were based on digits 1 and 5 and the wrist; data presented here for *T. scripta* were recalculated using the same landmarks applied for *C. caretta* (i.e., digits 1 and 5 and the elbow). A high-drag orientation of the forefoot paddle with the palmar surface of the paddle directed opposite the direction of travel (and in the same direction as the flow of water) is indicated by a feathering angle of 90°, and a perfect low-drag orientation of the forefoot paddle is indicated by a feathering angle of 0°.

humeral depression in *Caretta* (-51±2.6° versus -8±0.6° in *Trachemys*; Fig. 3.3A, Table 3.1). Maximum humeral elevation does not differ significantly between the species, though the humerus of *Trachemys* is held primarily above the horizontal plane, while that of *Caretta* is primarily below it (Fig. 3.3A, Table 3.1).

Peak humeral protraction (Fig. 3.3B) differs significantly between *Caretta* (64 ± 2.2°) and *Trachemys* (115±1.4°, Table 3.1). In addition, the humerus of *Caretta* is retracted far less (to an angle 26±2.0° anterior to the transverse axis, for an excursion averaging 38 ± 2.4°) than that of *Trachemys* (to an angle only 8±0.8° anterior to the transverse axis for an excursion averaging 107 ± 1.7°; Fig. 3.3B, Table 3.1). Thus, the range of anteroposterior motion of the humerus in rowing (*Trachemys*) is much greater than that observed in *Caretta* during flapping (Fig. 3.3B, Table 3.1). In fact, just as the humerus of *Trachemys* is held in a very narrow dorsoventral range of motion (Fig. 3.3A, Table 3.1), so too is the humerus of *Caretta* greatly restricted in its range of anteroposterior motion (Fig. 3.3B, Table 3.1). Despite these differences in the degree of humeral protraction and retraction between the species, they do not differ significantly in the timing of maximum humeral protraction (*Caretta*: 44±2.9% and *Trachemys*: 43±0.6% of the limb cycle; Fig. 3.3B, Table 3.1). Peak humeral protraction in *Caretta* is roughly coincident with the switch from elevation to depression, meaning that both species tend to reach peak humeral elevation temporally close to when they reach peak humeral protraction.

The pattern of elbow extension differs between *Caretta* and *Trachemys* (Fig. 3.3C). *Caretta* extends the elbow throughout the period of humeral elevation and protraction, reaching a single peak shortly after the start of humeral depression and retraction, at which point the elbow is flexed for the remainder of the limb cycle until it returns to its starting point (Fig. 3.3C). In contrast, swimming *Trachemys* flex the elbow for the first half of protraction (and elevation) and then begin elbow extension, reaching maximum extension midway through retraction (and depression), and then flexing the elbow for the remainder of the limb cycle to return to the starting position (Fig. 3.3C). It appears that the patterns are quite similar, though shifted temporally approximately a quarter-cycle out of phase. *Caretta* holds the elbow much straighter (more extended) than *Trachemys* throughout the limb cycle (Fig. 3.3C). While the species differ in the maximum degree of elbow extension, as well as flexion, they do not differ in the observed range of elbow motion or the percentage of the limb cycle at which maximum extension is achieved (Fig. 3.3C, Table 3.1).

The orientation of the forefoot relative to the direction of travel (or the direction of water flow) also differed between *Caretta* and *Trachemys* (Fig. 3.3D). This variable indicates whether the forefoot is in a high drag orientation (perpendicular to the direction of travel), or a low drag (feathered) orientation (Pace et al., 2001). Data presented here for forefoot orientation in both species are based on position of the digits and the elbow. This differs slightly from calculations and plot of this variable presented in Chapter 2 because I felt it best to compare this variable between the species once it had been calculated in the same manner. The forefoot of *Caretta* is held in an increasingly low-drag orientation throughout the first half of the limb cycle; at the start of humeral depression *Caretta* begins to rotate the forelimb towards a high-drag orientation (higher forefoot angles), reaching a peak mid-way through the downstroke, before returning to a lower-drag orientation (Fig. 3.3D). In contrast, *Trachemys* shows results similar to those published previously (Pace et al., 2001; see Chapter 2), in which the forefoot is feathered in a low-drag orientation in early protraction and reaches a high-drag peak (forefoot nearly perpendicular to the flow of water) very near the end of protraction; a second high-drag peak follows roughly two-thirds through the retraction phase (Fig. 3.3D), and ends with the palmar surface of the forefoot directed dorsally. While the general pattern of forefoot orientation differs between the species in that *Caretta* exhibits only a single peak, versus two for *Trachemys*, they are similar in that the forelimb is directed into a low-drag orientation during the first phase of the limb cycle, followed by a shift towards higher-drag orientation during the second phase of the limb cycle (Fig. 3.3D). While the two species do not differ in the total range of forefoot excursion or in the minimum degree of forefoot feathering (i.e., the lowest-drag orientation achieved), *Trachemys* feathers the forefoot less so that it is in a significantly higher drag position than experienced by the forefoot in *Caretta* (Table 3.1).

In summary, there are a number of strong differences between flapping and rowing kinematics for these species of swimming turtles (Table 3.1). Flapping in *Caretta* is characterized by a large range of dorsoventral humeral motion and a restricted amount of anteroposterior movement. In contrast, rowing in *Trachemys* is typified by a large amount of anteroposterior motion and limited dorsoventral movement. The greater dorsoventral range of motion during flapping is accomplished through an increase in humeral depression, but without a change in humeral elevation. The timing of maximum humeral elevation differs between the species, but within each species is roughly coincident with the timing of maximum protraction. The greater range of anteroposterior motion observed in rowing is achieved through both greater humeral protraction and retraction. Although the amount of elbow motion (excursion angle) is similar between the species, their elbows move through different arcs, with *Caretta* consistently holding the elbow in a more extended position. Finally, rowing in *Trachemys* is characterized by a much higher-drag orientation of the forefoot during the second phase of the limb cycle.

Patterns of muscle activation: flapping versus rowing

Among predicted humeral retractors and depressors, the coracobrachialis exhibits a single burst of activity during most of humeral depression and retraction in both *Caretta* and *Trachemys*, the timing of which does not differ between the species (Fig. 3.4, Table 3.2). In contrast, the other predicted humeral retractor, pectoralis, exhibits one burst of activity in *Caretta*, but presents two bursts of activity in *Trachemys* (Fig. 3.4). The early burst of pectoralis activity in *Trachemys* is variable (see Chapter 2) and, when present, always occurs during protraction/elevation. In contrast, the later burst of pectoralis activity in *Trachemys* always occurred during retraction/depression, similar to the single burst observed for *Caretta* (Fig. 3.4). The single burst in *Caretta* was compared to each of the two bursts of activity seen in *Trachemys* (Table 3.2). It differed significantly in both onset and offset of activity when compared with the *Trachemys* (variable) early burst; when compared to the later burst, it only differed in offset (Table 3.2).

Among humeral protractors and elevators, one muscle (latissimus dorsi) again exhibits a similar pattern between the species, but another (deltoideus) differs substantially (Fig. 3.4). In both species, the latissimus dorsi shows one long continuous burst of activity, starting shortly before the end of retraction and elevation and continuing into protraction and elevation (Fig. 3.4). Because my definition of the limb cycle divides these continuous bursts into two portions for graphic presentation, I term these portions as “early” and “late bursts” or “Burst

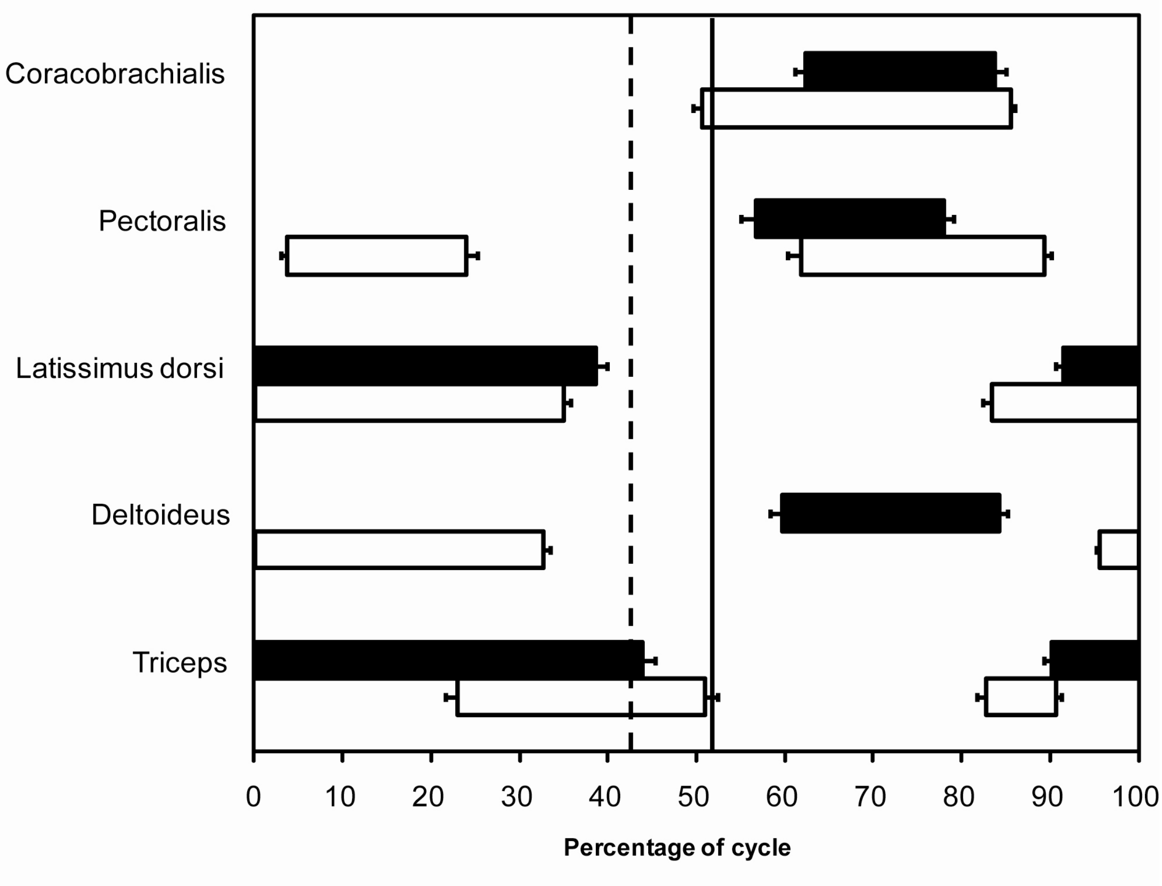

Fig. 3.4. Bar plot showing patterns of forelimb muscle activation during swimming for *C. caretta* and *T. scripta*. Data for *T. scripta* provided in Chapter 2. Bars represent the mean and standard error for the period of activity for each muscle. Solid bars represent flapping-style swimming of *C. caretta*, open bars represent rowing-style swimming in *T. scripta*. Vertical lines demarcate the switch from elevation to depression in *C. caretta* (solid line) and protraction to retraction in *T. scripta* (dashed line). The x-axis shows the percent of the limb cycle from 0 to 100%.

1” and “Burst 2” as in Chapter 2 to separate these descriptors from those for non-continuous bursts of activity in other muscles. Thus, for a continuous burst, such as exhibited by latissimus dorsi, onset refers to the beginning of activity observed for “Burst 2” and offset refers to the end of activity observed for “Burst 1”. The onset of “Burst 1” and the offset of “Burst 2” always occur at 0% and 100% of the limb cycle, respectively. Timing of activity for latissimus dorsi does not differ between *Caretta* and *Trachemys* (Table 3.2).

The deltoideus, the other focal humeral protractor and elevator, displays a very different pattern between the two study species (Fig. 3.4). *Caretta* shows just one discrete burst of deltoideus activity, occurring during depression and retraction (Fig. 3.4). In contrast, *Trachemys* shows one long continuous burst of deltoideus activity, starting just prior to the end of retraction and depression and continuing through most of protraction and elevation (Fig. 3.4). Onset of deltoideus activity in *Trachemys* is the start of “Burst 2” and offset is the end of “Burst 1 (see Chapter 2). To facilitate comparison of offset of deltoideus activity, offset in *Caretta* was transposed by subtracting 100. Comparisons of timing variables indicate significant differences in burst onset and offset, but not the duration of activity (Table 3.2).

The triceps complex also shows different patterns of activation between the two species. *Caretta* is characterized by a single continuous burst, starting near the end of depression and retraction and continuing through much of elevation and protraction (Fig. 3.4); this corresponds with elbow extension (Fig. 3.3C). *Trachemys*, however, exhibits two bursts of triceps activity; one burst straddles the switch from protraction/elevation to retraction/depression and the other occurs during the retraction/depression phase of the limb cycle (Fig. 3.4). While the early triceps burst was always present in *Trachemys*, the later burst was variable (see Chapter 2). To facilitate statistical comparisons between the species, onset of triceps activity in *Caretta* is the start of “Burst 2” and offset is the end of “Burst 1”; onset of the first burst in *Trachemys* was transposed by adding 100, whereas offset of the variable second burst was transposed by subtracting 100 (Table 3.2). Triceps activity in *Caretta* starts significantly earlier and has a significantly longer duration than the first burst of activity seen in *Trachemys*, though offset did not differ (Fig. 3.4, Table 3.2). Offset of triceps activity in *Caretta* occurred significantly later and lasted longer than the variable second burst of *Trachemys*, but onset did not differ (Fig. 3.4, Table 3.2).

Discussion

Kinematic comparison of flapping and rowing

The primary difference between flapping and rowing styles of swimming noted in previous observations (Walker, 1971; Walker, 1973; Davenport et al., 1984; Renous and Bels, 1993; Walker and Westneat, 2000; Pace et al., 2001; Blob et al., 2008; Renous et al., 2008; see Chapter 2) is supported by the details and the trends in my data (Fig. 3.3A, B). Dorsoventral humeral motion is much greater in flapping, whereas anteroposterior motion is much greater during rowing. Humeral motions outside of these predominant planes are constrained for both species. Nonetheless, several other kinematic distinctions emerge between these swimming styles. For example, the greater dorsoventral range of humeral motion in *Caretta* is achieved through a much greater degree of humeral depression than is usual for *Trachemys*, whereas the larger anteroposterior range of motion in *Trachemys* is accomplished through both increased humeral protraction and retraction relative to *Caretta*. In an additional distinction between the species, the forelimb of *Trachemys* generally moves through most of its cycle held slightly above the horizontal. In contrast, *Caretta* generally hold the forelimb depressed relative to the horizontal, with the majority of dorsoventral movement occurring below this plane (Fig. 3.3A). While differences in the orientation of the pectoral girdle between the species, as well as humeral head and process shape (Walker, 1973), may contribute to some of these differences in limb motion and (particularly) average limb orientation, the differences in muscle activation between the species suggest that structural differences are not the sole factor leading to the distinct humeral movements of rowing and flapping across turtles.

Elbow kinematics also differ between the species (Fig. 3.3C). Rowing, in *Trachemys,* is accomplished with a limb that that is first flexed and then extended at the elbow, before being flexed again. Flapping, in *Caretta,* is achieved by first extending the limb at the elbow, and flexing at the start of the downstroke while the flipper is depressed and retracted (Fig. 3.3C). While the angular excursion of the elbow does not differ between species, maximum extension and flexion do differ because the forelimb of *Caretta* is more fully extended (i.e., held straighter) throughout the limb cycle (Fig. 3.3C, Table 3.1). As for the humerus, while morphological differences between species (Walker, 1973) might contribute to the different orientations in which their elbows are held, differences in muscle activation observed between the species likely contribute to the differing phases of elbow motion that they exhibit.

Forefoot or flipper blade orientation shows both similarities and differences between the species. While total forefoot feathering excursion does not differ, a higher-drag orientation of the forefoot is observed in rowing *Trachemys* (Fig. 3.3D, Table 3.1), consistent with characterizations of these species as “drag-based” versus “lift-based” swimmers (e.g., Vogel, 1994; Wyneken, 1997). Yet, both hold the forefoot in a feathered (low-drag) orientation early in the limb cycle and then switch to a less feathered (higher-drag) orientation near the start of the second phase of the limb cycle. This cyclic reorientation of the flipper blade during swimming by sea turtles may help to maintain an appropriate angle of attack to allow the generation of thrust on both upstroke and downstroke (Vogel, 1994; Walker and Westneat, 2000). Evolution of the derived trait of flapping (Fig. 1.1) propulsion thus involved changes in a wide range of kinematic features beyond the primary plane of humeral motion (Licht et al., 2010).

Patterns of muscle activation during flapping-style swimming in Caretta

Four of the five pectoral girdle target muscles in *Caretta* were active during portions of the limb cycle as predicted based on their anatomical positions. The coracobrachialis and the pectoralis were active during humeral depression and retraction, the latissimus dorsi was active during humeral elevation and protraction, and the triceps complex was active during elbow extension (Fig. 3.4). However, the deltoideus was found to exhibit one discrete burst of activity during humeral depression and retraction; this is exactly opposite of when it was predicted to be active based on its anatomical position (Walker, 1973). Although the deltoideus was predicted to act primarily as a humeral protractor and elevator in sea turtles (Walker, 1973), its primary role is more likely as a modifier constraining humeral retraction during depression. *Caretta* show limited protraction during humeral elevation, and activity of the latissimus dorsi (a protractor and elevator) may be sufficient to produce this motion. Additionally, while the configuration of the pectoral girdle musculature is quite similar in all turtles (Walker, 1973), sea turtles (including *Caretta*) possess an enlarged pectoralis relative to that of freshwater turtles (Walker, 1973; Wyneken, 2001). The larger pectoralis of *Caretta* likely contributes to its substantial humeral depression, and could retract the humerus. The simultaneous protraction generated by the deltoideus as it fires during the forelimb downstroke (Fig. 3.4) should restrict the degree of humeral retraction produced by the pectoralis, thereby resulting in depression of the humerus with very little anteroposterior movement (Fig. 3.3B).

Are patterns of muscle activation conserved in the evolution of flapping?

With the majority of muscles active when predicted, based on their anatomical positions, it is not surprising that the comparison of swimming motor patterns in flapping *Caretta* and rowing *Trachemys* provide a composite of support for the ‘neuromotor conservation hypothesis’ and also data suggesting novel phenotypes have arisen. Among the conserved neuromuscular patterns is that of the coracobrachialis, one of the largest pectoral muscles in both species; it is active during retraction/depression in both species with no significant differences in timing. Similarly, the latissimus dorsi also displays a similar pattern of activity during elevation and protraction in both species, with no significant differences in timing. Thus, despite the dramatic differences in how *Caretta* and *Trachemys* swim, these two muscles display conserved patterns of activity, lending support to the hypothesis of neuromotor conservation.

In contrast, the pattern of activation for other muscles shows some marked differences that suggest a lack of conservation. This comparison reveals differences not only in the timing of muscle bursts (deltoideus), but also in the number of bursts (pectoralis and triceps complex) (Fig. 3.4). The deltoideus shows a dramatic shift in the timing of activity between rowing and flapping species that likely reflects a new role for this muscle in flapping swimming. While the deltoideus serves as a strong humeral protractor during rowing-style swimming of *Trachemys*, in *Caretta* it stabilizes and minimizes anteroposterior movements of the humerus through simultaneous activation with pectoralis. This activity could help to counter potential retraction generated by pectoralis during the downstroke of sea turtles, resulting in depression of the forelimb with limited anteroposterior movement during flapping. Thus, through a simple shift in activation timing, the functional role of deltoideus changes significantly for flapping swimmers. Clearly it was not conserved during the evolution of this locomotor behavior.

Similarly, pectoralis activity also shows a lack of conservation between rowing and flapping turtles, but through a more complicated set of differences. While *Trachemys* shows two discrete bursts of activity (one variable burst during elevation/protraction that may help to stabilize the shoulder, and a second during depression/retraction to draw the arm down and back), *Caretta* exhibits a single burst of pectoralis activity during depression/retraction, consistent with predictions based on its anatomical position (Fig. 3.4). Comparisons of the two bursts in *Trachemys* to the single burst of *Caretta* show significant differences in timing relative to the first burst (Table 3.2), but strong similarity to the second, with no differences in onset or duration, and only a slightly significant difference in timing of offset. Thus, pectoralis activity in *Caretta* appears to be conserved and homologous to the second burst of activity in *Trachemys*, but not the first. Why does *Caretta* not display the same variable pectoralis burst thought to act in shoulder stabilization in *Trachemys*? Flapping swimming is characterized by much less humeral protraction and much more humeral depression than rowing. However, the portion of the limb cycle in which pectoralis Burst 1 of *Trachemys* occurs (during slight elevation and protraction) is coincident with the *Caretta* upstroke (elevation and slight protraction). The enlarged pectoralis of *Caretta* acts as a strong humeral depressor and so it is likely that activation of this strong depressor during upstroke would be functionally and energetically counterproductive. In this highly migratory species, for which energetic efficiency over long distances of travel would likely be advantageous, such activity is unlikely to persist. Although the primary pattern of activity for pectoralis is conserved in the evolution of flapping, the early burst of activity for joint stabilization was lost with the shift in the plane of forelimb motion.

Finally, the triceps complex differs in both timing and number of muscle bursts between flapping and rowing species. While *Trachemys* shows two bursts of triceps activity, *Caretta* shows one long continuous burst. The early triceps burst in *Trachemys* occurs during elbow extension and was always present, while the later burst was variable and may act in elbow stabilization (see Chapter 2). Although timing of onset was similar, triceps activity in *Caretta* differs significantly in offset and duration from the variable second burst in *Trachemys* (Table 3.2). When compared to the early burst in *Trachemys* (the burst playing a similar role in elbow extension), I found significant differences in onset and duration (Table 3.2). While the primary function of the triceps (elbow extension) is similar in both species, the substantial kinematic differences in the pattern of elbow extension between flapping and rowing (Fig. 3.3C) appear to be controlled by a difference in the pattern of activation.

I conclude that the evolution of flapping-style swimming in sea turtles, as exemplified by *Caretta caretta*, is a case of a new locomotor behavior being accomplished through changes in both structure of the forelimb as well as some changes in the pattern of activation of forelimb muscles. I found the activity of several muscles (coracobrachialis, late pectoralis burst, latissimus dorsi) to be conserved between the species, but one muscle, deltoideus, has taken on a new role in flapping *Caretta*. In addition, though the triceps complex functions similarly to extend the elbow in both species, elbow kinematics differ sufficiently between species to require dramatic differences in the timing of activity between them. Additionally, in the evolution of flipper-based flapping, some variable muscle activity patterns found in rowing species (such as the early pectoralis burst and the late triceps complex burst, both thought to act in joint stabilization) are lost. Thus, while this study provides partial support for the hypothesis of neuromotor conservation, it also identifies notable exceptions.

Examination of additional species likely will determine if motor activation patterns are similarly modified across a broader range of locomotor behaviors. While most freshwater turtles swim via anteroposterior rowing, there are differences in the specifics of their limb kinematics. For example, aquatic specialists such as softshell turtles exhibit forelimb movements even more restricted to a horizontal plane (Pace et al., 2001). Additionally, *Carettochelys insculpta*, the Australian pig-nose turtle, exhibits independently derived flapping locomotion and, thus, would provide an opportunity to examine convergent evolution of forelimb morphology and flapping-style swimming. Examination of such species provide natural “experiments” that will shed light on how new forms of locomotion evolve and provide additional tests of the neuromotor conservation hypothesis.

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CHAPTER FOUR

FORELIMB KINEMATICS DURING SWIMMING IN THE PIG-NOSED TURTLE, *CARETTOCHELYS INSCULPTA,* COMPARED WITH OTHER TAXA: ROWING VERSUS FLAPPING, CONVERGENCE VERSUS INTERMEDIACY

Abstract

Animals that swim using appendages do so by way of rowing and/or flapping motions. While often represented as discrete categories, rowing and flapping are more appropriately viewed as points along a continuum of possible limb motions. Because turtles possess a rigid shell that restricts the production of propulsive forces to the limbs, they provide an ideal system in which to examine limb-based locomotor kinematics; moreover, turtles display a range of locomotor styles and associated limb morphologies. *Carettochelys insculpta* is unusual in that it is the only freshwater species to have flippers and swim via synchronous motions of the forelimbs that appear to be dorsoventral flapping motions, characteristics evolved independently of sea turtles. I used high-speed videography to quantify forelimb kinematics in *C. insculpta* and a closely related, highly aquatic rower (*Apalone ferox*). Comparisons of my new forelimb kinematic data to data previously collected for a generalized freshwater rower (*Trachemys scripta*) and a flapping sea turtle (*Caretta caretta*) allow me to assess (1) forelimb kinematics within and between locomotor modes across turtle species in order to more precisely quantify and characterize the range of limb motions used by flappers versus rowers, and (2) how *Carettochelys insculpta* swims using synchronous forelimb motions, whether they can be classified as flappers, and whether they exhibit forelimb kinematics more similar to closely related rowing species or distantly related flapping sea turtles. I found that rowers are most similar to each other, and more similar to *Carettochelys* than to *Caretta*. “Flapping” in *Carettochelys* is achieved through very different humeral kinematics than in *Caretta*; nevertheless, of the three freshwater species, *Carettochelys* was most similar to flapping *Caretta*. My data support characterizing *Carettochelys* as a synchronous rower, although some kinematic parameters appear intermediate between rowing freshwater species and flapping marine species.

Introduction

Animals that propel themselves using appendages (e.g., fins or limbs), do so by way of rowing and/or flapping motions. Rowing is characterized by anteroposterior oscillatory motions of the limbs with distinct recovery and power strokes (Blake, 1979; Blake, 1980; Vogel, 1994; Walker and Westneat, 2000), whereas flapping is characterized by dorsoventral oscillatory motions of the limbs, in which a distinct recovery stroke may not be present (Aldridge, 1987; Rayner, 1993; Walker and Westneat, 1997; Wyneken, 1997; Walker and Westneat, 2000; Chapter 3). Aquatic locomotion via rowing and flapping has been reported for a diverse range of taxa, including invertebrates (Plotnick, 1985; Seibel et al., 1998), fishes (Walker and Westneat, 2000; Walker, 2002; Walker and Westneat, 2002a; Walker and Westneat, 2002b), turtles (Davenport et al., 1984; Pace et al., 2001; see Chapters 2 and 3), birds (Baudinette and Gill, 1985), and mammals (Feldkamp, 1987; Fish, 1993; Fish, 1996).

Rowing and flapping fishes, in particular, have provided a productive system in which to examine the functional consequences and correlates of these two methods of swimming. Flapping has been shown to be a more energetically efficient mode of swimming than rowing, regardless of swimming speed (Walker and Westneat, 2000). This suggests that flapping should be employed by species that require energy conservation (Walker and Westneat, 2000), such as those that swim great distances. However, rowing appendages were found to generate more thrust during the power stroke, and to be better for maneuvers such as accelerating, braking, and turning (Walker and Westneat, 2000), suggesting that species that live in aquatic environments that require substantial maneuvering should employ rowing. A strong correlation between swimming mode and limb morphology also exists, with rowing appendages typically distally expanded or paddle shaped and flapping appendages typically distally tapering and wing-shaped (Walker, 2002; Walker and Westneat, 2002a; Walker and Westneat, 2002b). A further pattern associated with this dichotomy in swimming modes is that many rowing species are not fully aquatic like fishes, but instead semi-aquatic. Semi-aquatic animals must function effectively on land, as well as in water, and limbs suited for rowing are better suited for terrestrial locomotion than those used for flapping (Vogel, 1994; Fish, 1996; Walker and Westneat, 2000). Moreover, animals for which forelimbs have evolved into specialized foreflippers used in aquatic flapping are rarely adept at terrestrial locomotion (e.g., pinnipeds) (Feldkamp, 1987; Renous and Bels, 1993; Fish, 1996).

Although the qualitative difference between rowing and flapping as modes of aquatic propulsion is well established, empirical quantification of the kinematic distinctions between these locomotor styles for comparisons across species has been rare. Such quantitative comparisons would be particularly useful for lineages in which these styles have arisen multiple times, as these data could aid understanding of evolutionary diversification in locomotor function and the nature of functional transitions (e.g., gradual versus abrupt) in such groups. In this context, turtles provide an ideal system in which to compare aquatic propulsion via oscillatory motions of appendages. As a result of their immobilized axial skeleton and reduced tail, thrust in swimming turtles is generated exclusively by the movements of forelimbs and hindlimbs (Zug, 1971; Wyneken, 1997; Pace et al., 2001; Blob et al., 2008; Rivera et al., 2011). Thus, evaluations of differences in swimming kinematics across taxa are not confounded significantly by the contributions of other structures to propulsion, like flexible bodies, tails, or specialized fins (Blake et al., 1995; Walker, 2000; Fish, 2002; Fish and Nicastro, 2003; Rivera et al., 2006).

While there are many differences among species of aquatic turtle (>200 species) with regard to their locomotion in aquatic habitats (Webb, 1962; Zug, 1971; Walker, 1973; Davenport et al., 1984; Pace et al., 2001; Blob et al., 2008; Renous et al., 2008), one of the most striking examples is in the use of rowing versus flapping in swimming taxa. Asynchronous rowing is the more common and ancestral form of swimming in turtles (Joyce and Gauthier, 2004) and has been reported to be used exclusively by all but one freshwater species (Fig. 1.1). In rowing turtles, the forelimb of one side moves essentially in phase with the contralateral hindlimb, so that forelimbs (and hindlimbs) of opposite sides move asynchronously (Pace et al., 2001; Rivera et al., 2006; Rivera et al., 2011; see Chapter 2). Rowing species also tend to possess moderate to extensive webbing between the digits of the forelimb and hindlimb (Pace et al., 2001) [i.e., distally expanded and paddle-shaped; (Walker and Westneat, 2002a)]. Synchronous flapping is a much rarer locomotor style used by turtles, definitively employed by the seven extant species of sea turtle (Wyneken, 1997; Fig. 1.1). Flapping turtles swim via synchronous motions of forelimbs that have been modified into flat, elongate, semi-rigid flippers [i.e., distally tapering wing-like appendages; (Walker and Westneat, 2002a)]. Foreflippers may produce thrust on both upstroke and downstroke, but the hindlimbs have a negligible propulsive role (Walker, 1971; Walker, 1973; Davenport et al., 1984; Renous and Bels, 1993; Walker and Westneat, 2000). In addition, a single freshwater species, the pig-nosed turtle *Carettochelys insculpta*, is described as using synchronous flapping-style motions to swim (Walther, 1921; Rayner, 1985; Georges et al., 2000; Walker, 2002), which would represent an independent convergence on this swimming style within the chelonian lineage. *Carettochelys insculpta* is the sole extant member of the carettochelyid lineage that forms the sister taxon to the trionychid clade (Fig. 1.1) (Engstrom et al., 2004; Fujita et al., 2004; Iverson et al., 2007; Barley et al., 2010). While trionychids are highly specialized rowers with extensive webbing between the digits of the forelimb (Pace et al., 2001), this morphology appears even further hypertrophied in *C. insculpta* through elongation of both the digits and webbing, so that the forelimbs of this species converge on at least a superficial resemblance to the foreflipper anatomy of sea turtles (Walther, 1921). Yet, while described as using flapping forelimb motions (Rayner, 1985; Ernst and Barbour, 1989; Georges et al., 2000), kinematic measurements from *C. insculpta* are not currently available that would allow quantitative comparisons with flapping by sea turtles and evaluations of the similarity of these purportedly convergent locomotor styles.

Although descriptions of appendicular motions during swimming are commonly framed dichotomously as either rowing or flapping, these characterizations may be more correctly viewed as extremes along a continuum of possible limb motions (Gatesy, 1991; Carrano, 1999; Walker and Westneat, 2002a). Understanding appendicular swimming kinematics beyond just the predominant plane of motion (i.e., anteroposterior versus dorsoventral) would allow for a better understanding of whether suites of kinematic parameters (e.g., humeral and elbow kinematics, forefoot feathering) in turtles can rightfully be described as “rowing” or “flapping”. Although summaries of patterns of forelimb motion have been reported for some species of turtle (Walker, 1971; Davenport et al., 1984; Renous and Bels, 1993; Wyneken, 1997), detailed kinematic data from the forelimb during swimming are available for only a few species of turtle, including rowing by the emydid *Trachemys scripta* [red-eared slider; (Pace et al., 2001; see Chapter 2)] and the trionychid *Apalone spinifera* [spiny softshell; (Pace et al., 2001)], and flapping employed by *Caretta caretta* [loggerhead sea turtle; (see Chapter 3)]. Among rowers, there are some notable kinematic differences between the semi-aquatic generalist *T. scripta*, a lentic species which spends considerably more time out of water than the lotic, aquatic specialist *A. spinifera*; in particular, the aquatic specialist greatly restricts the range of anteroposterior (less than half that of *T. scripta*) and dorsoventral (less than a third that of *T. scripta*) motions of the forelimb (Pace et al., 2001). These findings indicate that in addition to differences in kinematics between modes of locomotion (i.e., flapping vs. rowing), significant variation can also exist within locomotor modes.

The goals of this study were to (1) examine forelimb kinematics within and between locomotor modes across turtle species to more precisely quantify and characterize the range of limb motions used by flappers versus rowers and (2) determine how *Carettochelys insculpta* uses synchronous forelimb movements to swim and whether phylogenetic similarity or locomotor mode (i.e., synchronous swimming using foreflippers, commonly described as flapping) correlates more strongly with the kinematics displayed by this distinctive freshwater species. To address these questions, I quantified forelimb kinematics during swimming by pig-nosed turtles (*C. insculpta* Ramsay, 1886) and rowing Florida softshell turtles (*Apalone ferox* Schneider, 1783), and compared these results to data from two additional species: my previous measurements of forelimb kinematics from the slider [*Trachemys scripta* Schoepff, 1972; (see Chapter 2)] and loggerhead sea turtle [*Caretta caretta* Linnaeus, 1758; (see Chapter 3)], representing generalized rowing and characteristic flapping, respectively. *Apalone ferox* is an aquatic specialist and member of the sister group to *C. insculpta*; unlike other *Apalone* species [such as the previously studied *A. spinifera* (Pace et al., 2001)], *A. ferox* prefers the lentic conditions of lakes and ponds rather than lotic rivers and, when found in rivers, usually prefers the slower portions (Ernst and Lovich, 2009). As such, *A. ferox* may provide a more appropriate comparison to *C. insculpta* [which also prefers slow currents; (Ernst and Barbour, 1989; Georges et al., 2000; Georges and Wombey, 2003)], than *A. spinifera*. Furthermore, data from *A. ferox* will also provide an additional point of comparison among the diversity of rowing species, and as a lentic species provides an important comparison to *T. scripta*. Moreover, swimming in *C. insculpta* is typically described as being similar to that of sea turtles, however, quantified kinematic data from swimming by this unusual species are not currently available for comparison. These comparisons will allow me to evaluate the extent to which carettochelyids and sea turtles have converged on similar flapping kinematics, or whether aspects of forelimb kinematics in *C. insculpta* bear closer resemblance to the motions of their close relatives like *A. ferox.*

Materials and Methods

Experimental animals

Access to turtles was provided by a commercial vendor (Turtles and Tortoises Inc., Brooksville, FL, USA). Data were collected from two *Carettochelys insculpta* (carapace length = 23.8±1.8 cm) and nine *Apalone ferox* (carapace length = 15.1±1.1 cm). The number and size of *C. insculpta* was limited due to highly infrequent availability of this rare species. Turtles were housed in 600 liter (150 gallon) stock tanks equipped with pond filters; *A. ferox* were provided with dry basking platforms. Tanks were located in a temperature-controlled greenhouse facility, thus exposing turtles to ambient light patterns during the course of experiments. *Carettochelys insculpta* were fed a diet of commercially available algae wafers (Hikari®, Hayward, CA, USA) and fresh kiwi and bananas. *A. ferox* were fed a diet of commercially available reptile food (ReptoMin®, Tetra®, Blacksburg, VA, USA), supplemented with earthworms. All animal care and experimental procedures were conducted in accordance with Clemson University IACUC guidelines (protocols 50110, 2008-013, and 2008-080). Experimental procedures followed those of my previous studies of slider turtles (see Chapter 2) and sea turtles (see Chapter 3) as closely as possible to facilitate comparisons among the four species.

Collection and analysis of kinematic data

Kinematic data from swimming *C. insculpta* and *A. ferox* were collected simultaneously in lateral and ventral views (100 Hz) using two digitally synchronized high-speed video cameras (Phantom V4.1, Vision Research, Inc.; Wayne, NJ, USA). Locomotor trials for *C. insculpta* were conducted in a glass aquarium and those for *A. ferox* were conducted in a custom-built recirculating flow tank with a transparent glass side and bottom (see Appendix F). Ventral views were obtained by directing the ventral camera at a mirror oriented at a 45° angle to the transparent bottom of the tank. Swimming trials were collected from each turtle, yielding 17 and 22 cycles from each *C. insculpta* and 20-25 limb cycles from each *A. ferox.* For *A. ferox*, water flow was adjusted to elicit forward swimming behavior (Pace et al., 2001; see Chapter 2); once the turtle was swimming, flow was adjusted to keep pace with the swimming speed of the animal so as it remained in the field of view of the cameras. As *C. insculpta* would not readily swim in flow, and because it was necessary for turtles to stay in the field of view of the camera for several consecutive limb cycles, the posterior marginal scutes of *C. insculpta* were gently held, restricting forward movement of the animal while eliciting normal swimming motions of the limbs. Validity of this method was supported by the lack of a significant difference in the values of kinematic variables (N=8, see statistical analysis below) compared between free-swimming (N=4) and restrained (N=17) trials for one individual (MANOVA: Wilks lambda = 0.386; F = 2.389; d.f. = 8, 12; P = 0.084).

To facilitate digitization of animal movement from videos, a combination of white correction fluid and black marker pen were used to draw high-contrast points on the following 13 anatomical landmarks (Fig. 4.1): tip of the nose; shoulder; elbow; wrist (*A. ferox* only); digits 1, 3, and 5; an anterior and posterior point on the bridge of the shell (visible in lateral and ventral view); and right, left, anterior, and posterior points on the plastron (plastral points visible in ventral view only). Landmark positions were digitized frame-by-frame in each video using DLTdataViewer2 (Hedrick, 2008). The three-dimensional coordinate data generated were then processed using custom Matlab (Student Ver. 7.1, MathWorks, Inc.; Natick, MA, USA) routines to calculate limb kinematics during swimming, including protraction and retraction of the humerus, elevation and depression of the humerus, extension and flexion of the elbow, forefoot orientation angle, and displacement of the tip of digit 3 in the anteroposterior and dorsoventral directions. Calculated values for kinematic variables from each limb cycle were fit to a quintic spline (Walker, 1998) to smooth the data, and interpolated to 101 values, representing 0 through 100 percent of the limb cycle. Transformation of the duration of each cycle to a percentage allowed me to compare locomotor cycles of different absolute durations and calculate average kinematic profiles and standard errors for each variable through the course of the limb cycle. A humeral protraction/retraction angle of 0° indicates that the humerus is perpendicular to the midline of the turtle, while an angle of 90° indicates a fully protracted forelimb with the distal end of the humerus directed

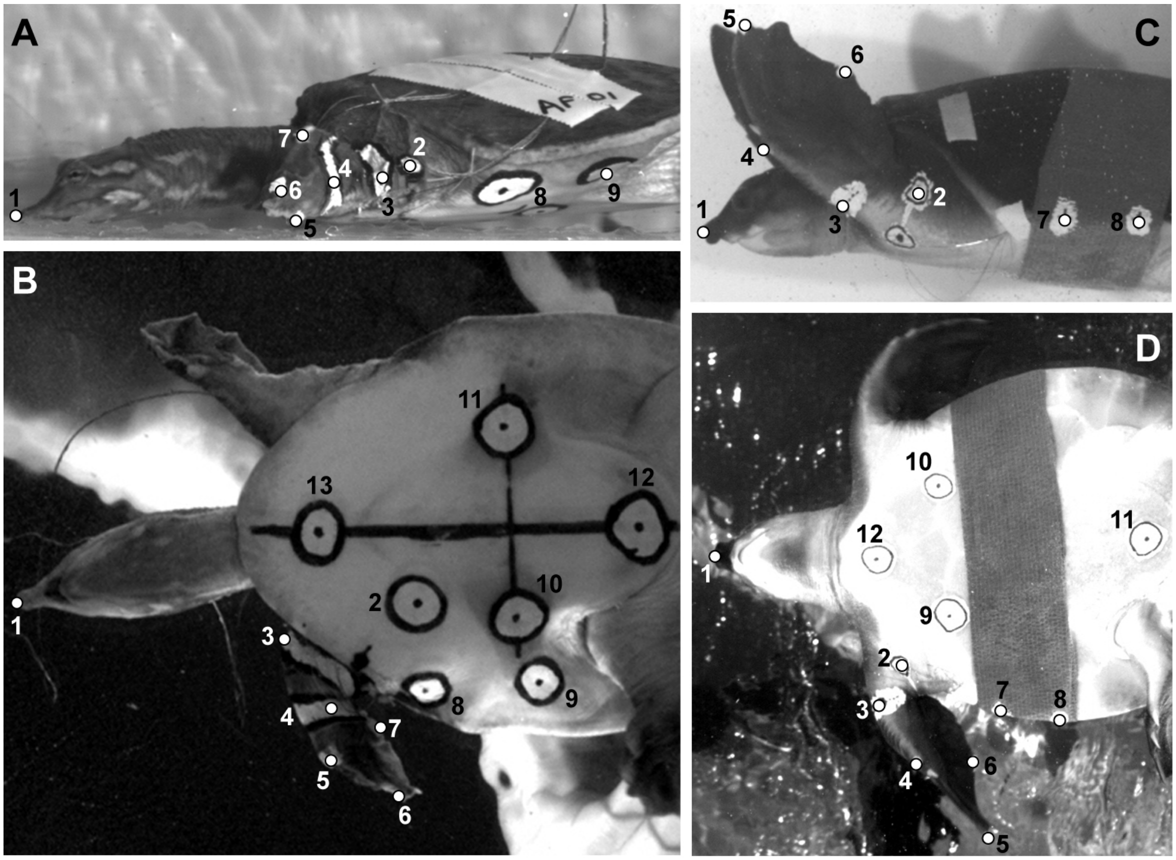

Fig. 4.1. Representative still images from lateral and ventral videos showing landmarks digitized for kinematic analysis of *Apalone ferox* and *Carettochelys insculpta*. (AB) *Apalone ferox*: Points 1-9 are the same in lateral and ventral view; points 10-13 are only visible in ventral view. Landmarks include: 1- tip of the nose, 2- shoulder, 3- elbow, 4-wrist, 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, and 13-anterior point on plastron. (CD) *Carettochelys insculpta*: Points 1-8 are the same in lateral and ventral view; points 9-12 are only visible in ventral view. Landmarks include: 1- tip of the nose, 2- shoulder, 3- elbow, 4-digit 1, 5-digit 3, 6-digit 5, 7-anterior point on bridge, 8-posterior point on bridge, 9-point on left side of plastron, 10-point on right side of plastron, 11-posterior point on plastron, and 12-anterior point on plastron

anteriorly (an angle of -90° would indicate a fully retracted forelimb with the distal tip of the humerus directed posteriorly). A humeral elevation/depression angle of 0° indicates that the humerus is in the horizontal plane. Angles greater than zero indicate elevation above the horizontal (distal end above proximal end) while negative angles indicate depression of the humerus (distal end lower than proximal end). Extension of the elbow is indicated by larger extension/flexion angles and flexion is indicated by smaller values. An elbow angle of 0° indicates the hypothetical fully flexed (i.e., humerus perfectly parallel to radius and ulna) elbow, 180° indicates a fully extended elbow, and 90° indicates that the humerus is perpendicular to the radius and ulna. Forefoot orientation angle was also calculated as the angle between a vector pointing forwards along the anteroposterior midline (also the path of travel) and a vector emerging from the palmar surface of a plane defined by the tips of digits 1 and 5 and the elbow (*C. insculpta*) or wrist (*A. ferox*); this angle was transformed by subtracting 90° from each value (Pace et al., 2001; see Chapters 2 and 3). A high-drag orientation of the forefoot paddle with the palmar surface of the paddle directed opposite the direction of travel (and in the same direction as the flow of water) is indicated by an angle of 90°, and a perfect low-drag orientation of the forefoot paddle is indicated by an angle of 0°.

Statistical analysis

To assess general patterns of movement, the overall mean and standard error of each variable was calculated for all swimming trials. Kinematic variables include: (i) maximum protraction, retraction, elevation, and depression of the humerus, (ii) maximum elbow extension and flexion, (iii) anteroposterior and dorsoventral excursion of the humerus, (iv) elbow excursion, (v) percentage of the cycle at which maximum elbow extension occurs, (vi) the percentage of the limb cycle at which a switch from protraction to retraction occurs, (vii) the maximum, minimum, and range of feathering of the forefoot, and (viii) the ratio of dorsoventral to anteroposterior excursion of the tip of digit 3. Because the maximum values for each limb cycle do not always occur at the same percentage of the limb cycle, it is possible that the average of the maximum values calculated for all limb cycles may be masked (appear lower) in average kinematic profiles. I compare my data for *C. insculpta* and *A. ferox* to that previously published for rowing-style swimming in the generalized freshwater slider *T. scripta* (see Chapter 2) and flapping-style swimming in loggerhead sea turtles (*C. caretta*) (see Chapter 3). I used SYSTAT 13 (Systat Software, Inc., Chicago, IL, USA) and R 2.12 (R Development Core Team, 2010) for statistical analyses, and $P<0.05$ as the criterion for significance.

To determine whether swimming kinematics differed overall among the four species, I conducted a two-way nested MANOVA, with species as a fixed factor and individual (nested within species) as a random factor. All multivariate analyses used standardized values (Z-scores) (Gotelli and Ellison, 2004) for 8 angular kinematic variables: maximum humeral protraction, retraction, elevation, and depression; maximum elbow extension and flexion; and maximum and minimum forefoot feathering. Excursions were not included in multivariate analyses because they are compositional data (i.e., the difference between minimum and maximum values), and as such are highly correlated with the variables used to calculate them. Next, kinematic differences were visualized using principal components analysis. While PCA can visually demonstrate the difference in kinematics among the species, it does not accurately illustrate the true multidimensional difference among them. To illustrate this more clearly, the Euclidean distances (D) between all pairs of species means were calculated using the 8 variables described above. To determine which pairs of species differed I used a permutation procedure (Adams and Collyer, 2009; Marsteller et al., 2009), in which the observed Euclidean distances between the least-squares means for the proper species-turtle assignments were compared to a distribution of possible values obtained by randomizing trial data among species-individual assignments. This randomization process was repeated 9999 times and the proportion of randomly generated values that exceeded the observed values was treated as the significance level (P_{rand}) (Adams and Collyer, 2007; Collyer and Adams, 2007; Adams and Collyer, 2009; Marsteller et al., 2009).

To evaluate differences among the species with respect to the 14 kinematic variables that characterize swimming in each, I conducted separate two-way mixed-model nested ANOVAs (corrected for unbalanced sampling), with species as a fixed factor and individual (nested within species) as a random factor. For each significant ANOVA, I conducted posthoc Tukey pair-wise mean comparison tests to determine which species pairs differed. In tabular data summaries, I provide d.f. and *F*-values to clarify the potential effects of making multiple comparisons.

Results

Herein I report new data on the kinematics of swimming in *Carettochelys insculpta* (39 cycles from 2 turtles) and *Apalone ferox* (195 cycles from 9 turtles); I compare my new data to previously published findings for *Trachemys scripta* [136 cycles from 7 turtles; (see Chapter 2)] and *Caretta caretta* [33 cycles from 3 turtles; (see Chapter 3)]. As for *C. insculpta*, the smaller number of individuals from which data were collected for *C. caretta* reflects their rare and threatened status (see Chapter 3). Kinematic plots depicting the general pattern of limb motion during swimming in each species were constructed using my new data for *Carettochelys* and *Apalone* and published data for *Trachemys* (see Chapter 2) and *Caretta* (see Chapter 3). Turtles of each species swam using similar forelimb cycle frequencies (*C. insculpta*: 1.78±0.06 cycles/sec; *A. ferox*: 2.24±0.03 cycles/sec; *T. scripta*: 2.29±0.04 cycles/sec; *C. caretta*: 1.85±0.05 cycles/sec). A summary of sample sizes from each individual, by species, is given for statistical analyses (see Appendix F).

Kinematics of swimming in Carettochelys insculpta *and* Apalone ferox

Limb motions in swimming *C. insculpta* are characterized by a threefold greater degree of anteroposterior humeral motion (97±1.8 deg) than dorsoventral motion (31±1.4 deg; Fig. 4.2A, B; Table 4.1). Hence, following previous conventions, a limb cycle in *C. insculpta* is defined similarly to that in rowing species, beginning at the start of humeral protraction and ending at the start of the next protraction cycle (Pace et al., 2001; Blob et al., 2008; see Chapter 2). Protraction in *C. insculpta* occupies slightly more than the first half (51±0.9%) of the limb cycle (Fig. 4.2A; Table 4.1). The humerus reaches a single peak of protraction (126±0.7 deg), followed by a return of the humerus to the retracted position (maximum retraction angle = 29±0.6 deg; Fig. 4.2A; Table 4.1). Throughout the limb cycle, the humerus of *C. insculpta* is held depressed relative to the horizontal, and displays a bimodal pattern of elevation and depression, reaching a first peak during protraction and a second peak during retraction (Fig. 4.2A, B). The elbow is at its most flexed position at the beginning and end of the limb cycle (92±1.3 deg). The elbow gradually extends throughout protraction, reaching a single peak of 128±0.8 deg at 49±1.2% of the limb cycle, approximately coincident with the timing of maximal humeral protraction (51±0.9%), followed by a return to the fully flexed position by the end of the cycle (Fig. 4.2C; Table 4.1). During the first ~10% of the limb cycle, the forefoot of *C. insculpta* is rotated into a low-drag, feathered orientation; the forefoot remains feathered throughout the recovery (i.e., protraction) phase (Fig. 4.2D; Table 4.1).

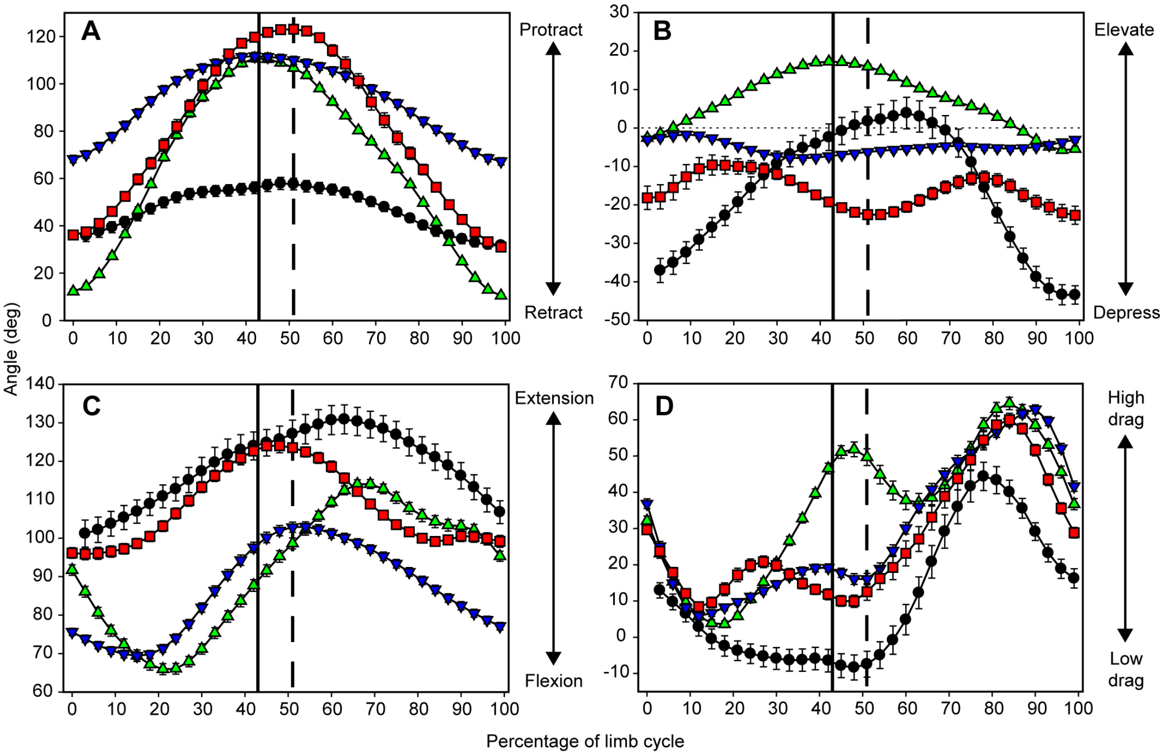

Fig. 4.2. Mean kinematic profiles of swimming in four species of turtle. Species included are *Carettochelys insculpta* (red squares), rowing *Apalone ferox* (inverted blue triangles), rowing *Trachemys scripta* (green triangles), and flapping *Caretta caretta* (black circles). Data for *T. scripta* provided in Chapter 2. Data for *C. caretta* provided in Chapter 3. Each trial was normalized to the same duration and angle values interpolated to represent 0-100% of the limb cycle. For *C. insculpta*, *A. ferox*, and *T. scripta*, the limb cycle is defined as protraction of the humerus followed by retraction; for *C. caretta*, the limb cycle is defined as elevation of the humerus followed by depression. Mean angle values ± S.E.M. are plotted for every third increment (every 3% through the cycle) for all individuals. Solid vertical lines demarcate the switch from protraction to retraction in *A. ferox* and *T. scripta* at 43% of the limb cycle. Dashed vertical lines indicate the switch from protraction to retraction in *C. insculpta* and from elevation to depression in *C. caretta* at 51% of the limb cycle. (A) Humeral protraction and retraction (i.e., angle from the transverse plane). An angle of 0° indicates that the humerus is perpendicular to the midline of the turtle, while an angle of 90° indicates a fully protracted forelimb with the distal end of the humerus directed anteriorly (an angle of -90° would indicate a fully retracted forelimb with the distal tip of the humerus directed posteriorly). (B) Humeral elevation and depression (i.e., angle from the horizontal plane). An angle of 0° indicates that the humerus is in the horizontal plane. Angles greater than zero indicate elevation above the horizontal (distal end above proximal end) and

negative angles indicate depression of the humerus (distal end lower than proximal end). Peak elevation is coincident with peak protraction for *Trachemys* and *Caretta*, meaning that limb protraction happens at the same time as elevation and retraction is concurrent with depression. (C) Elbow flexion and extension. Extension is indicated by larger angles and flexion is indicated by smaller angles. An angle of 0° indicates complete flexion, 180° indicates a fully extended elbow, and 90° indicates that the humerus is perpendicular to the radius and ulna. (D) Forefoot orientation angle is calculated as the angle between a vector pointing forwards along the anteroposterior midline (also the path of travel) and a vector emerging from the palmar surface of a plane defined by the tips of digits 1 and 5 and the elbow; this angle is transformed by subtracting 90° from each value. A high-drag orientation of the forefoot paddle with the palmar surface of the paddle directed opposite the direction of travel (and in the same direction as the flow of water) is indicated by a feathering angle of 90°, and a perfect low-drag orientation of the forefoot paddle is indicated by a feathering angle of 0°.

Concurrent with the start of humeral retraction (i.e., thrust phase), the forefoot is rotated into a high-drag orientation, nearly perpendicular to the direction of flow (67± 1.9 deg; Fig. 4.2D; Table 4.1). Maximum high-drag forefoot orientation is achieved near the end of the thrust phase, after which the forefoot is rotated back to a feathered orientation for the remainder of the swimming stroke.

Because *A. ferox* swims via rowing motions of the limbs, I follow the previously established convention of defining the limb cycle as starting at the beginning of humeral protraction and ending at the start of the next protraction cycle (Pace et al., 2001; Blob et al., 2008; see Chapter 2). The limb cycle can be divided into two separate phases; humeral protraction represents the “recovery” phase, followed by retraction of the humerus through the “thrust” phase. In *A. ferox*, humeral protraction comprises the first 43±0.6% (mean ± s.e.m.) of the

Table 4.1. Mean values and standard errors of humeral kinematic variables and F-values for the main effect of species from two-factor mixed model nested ANOVAs performed separately on each variable

Variable	*Apalone ferox*	*Trachemys scripta*	*Carettochelys insculpta*	*Caretta caretta*	*F*-value (d.f. 3,17)
Maximum humeral depression[1]	-11±0.6	-8±0.6	-32±1.4	-51±2.6	29.58***
Maximum humeral elevation[1]	2±0.7	20±0.7	-1±2.2	10±3.7	5.90**
Dorsoventral humeral excursion angle[3]	13±0.4	28±0.7	31±1.4	61±4.5	52.19***
Maximum humeral retraction[1]	64±1.5	8±0.8	29±1.6	26±2.0	20.07***
Maximum humeral protraction[1]	113±1.7	115±1.4	126±0.7	64±2.2	6.88**
% of limb cycle at maximum protraction[2]	43±0.6	43±0.6	51±0.9	44±2.9	3.11*
Anteroposterior humeral excursion angle[3]	49±1.3	107±1.7	97±1.8	38±2.4	25.59***
Maximum elbow flexion[1]	67±1.1	61±1.3	92±1.3	93±3.6	3.96*
Maximum elbow extension[1]	107±1.2	123±0.9	128±0.8	139±3.1	5.75**
% of limb cycle at maximum elbow extension[2]	56±0.8	68±1.3	49±1.2	59±4.0	5.07**
Elbow excursion angle[3]	40±1.0	62±1.5	36±1.1	46±3.3	4.37*
Maximum forefoot feathering[1]	76±1.0	78±1.1	67±1.9	54±3.1	6.15**
Minimum forefoot feathering[1]	-4±1.0	-5±1.2	-1±1.0	-18±3.0	2.50
Total Forefoot feathering excursion[3]	80±1.0	83±1.2	68±1.8	72±2.7	1.80
DV/AP excursion ratio of digit 3[4]	0.23±0.01	0.29±0.01	0.58±0.03	1.47±0.13	35.60***

[1] Values are angles in degrees

[2] Values represent a percentage of the limb cycle

[3] Values represent the total angular excursion

[4] Ratio of dorsoventral (DV) to anteroposterior (AP) excursions of distal-most point of the forelimb (digit 3)

**P*≤0.05; ***P*≤0.01; ****P*≤0.001; Data for *T. scripta* provided in Chapter 2. Data for *C. caretta* provided in Chapter 3.

limb cycle (Fig. 4.2A; Table 4.1). A single peak of humeral protraction (113±1.7 deg) is followed by a return of the forelimb to the retracted position (maximum retraction angle = 64±1.5 deg; Fig. 4.2A; Table 4.1). Throughout the limb cycle, the humerus of *A. ferox* shows very little elevation or depression, primarily being held at a slightly depressed angle relative to the horizontal plane (Fig. 4.2B). Hence, the range of anteroposterior humeral motion (49±1.3 deg) is far greater than the dorsoventral range (13±0.4 deg) (Fig. 4.2A, B; Table 4.1). The elbow flexes at the beginning of protraction, but then gradually extends throughout the remainder of protraction, reaching a single peak of 107± 1.2 deg at 56±0.8% of the limb cycle, followed by flexion (Fig. 4.2C; Table 4.1). During the first ~10% of the limb cycle, the forefoot of *A. ferox* is rotated into a low-drag, feathered orientation; the forefoot remains feathered throughout the recovery (i.e., protraction) phase (Fig. 4.2D; Table 4.1). Shortly after the start of humeral retraction (i.e., thrust phase), the forefoot is rotated into a high-drag orientation, nearly perpendicular to the direction of flow (76± 1.0 deg; Fig. 4.2D; Table 4.1). Maximum high-drag forefoot orientation is achieved near the end of the thrust phase, after which the forefoot is rotated back to a feathered orientation for the remainder of the swimming stroke.

Multi-species comparisons of the kinematics of rowing and flapping

Using nested MANOVA, I found significant differences in the kinematics of swimming among *C. insculpta*, *A. ferox*, *T. scripta*, and *C. caretta* (Wilks lambda

= 0.002; F = 8.74; d.f. = 24, 29; $P < 0.001$). Principle components analysis visually demonstrates the differences in overall swimming kinematics among these species (Fig. 4.3; see Table 4.2 for PC loadings). While the first two PC axes account for 56.9% of the total variation in angular forelimb kinematics among species, the true multidimensional difference among them is depicted more clearly by the pair-wise Euclidean distances between species means (Table 4.3). Listed from smallest to largest, these were: *Apalone-Trachemys*, *Apalone-Carettochelys*, *Trachemys-Carettochelys*, *Carettochelys-Caretta*, *Trachemys-Caretta*, and *Apalone-Caretta* (Table 4.3). All pair-wise species comparisons were found to be significant using permutation tests ($P_{rand} < 0.001$). Two-way nested ANOVAs showed significant differences among the species for 13 out of 15 kinematic variables; only minimum forefoot feathering and total forefoot feathering excursion angle were found to not differ (Table 4.1). Tukey pair-wise species comparison results for each significant ANOVA are given in Table 4.4.

While the predominant direction of humeral motion for all three freshwater species is anteroposterior, the range of motion in *A. ferox* (49±1.3 deg) and *C. caretta* (38±2.4 deg) is similarly small and differs significantly from that of *C. insculpta* (97±1.8 deg) and *T. scripta* (107±1.7 deg), which do not differ (Fig. 4.2A; Tables 4.1, 4.4). With its narrow anteroposterior range of humeral motion and a similar peak value of protraction to that of *C. insculpta* and *T. scripta*, *A. ferox* retracts the humerus significantly less than other species (Fig. 4.2A; Tables

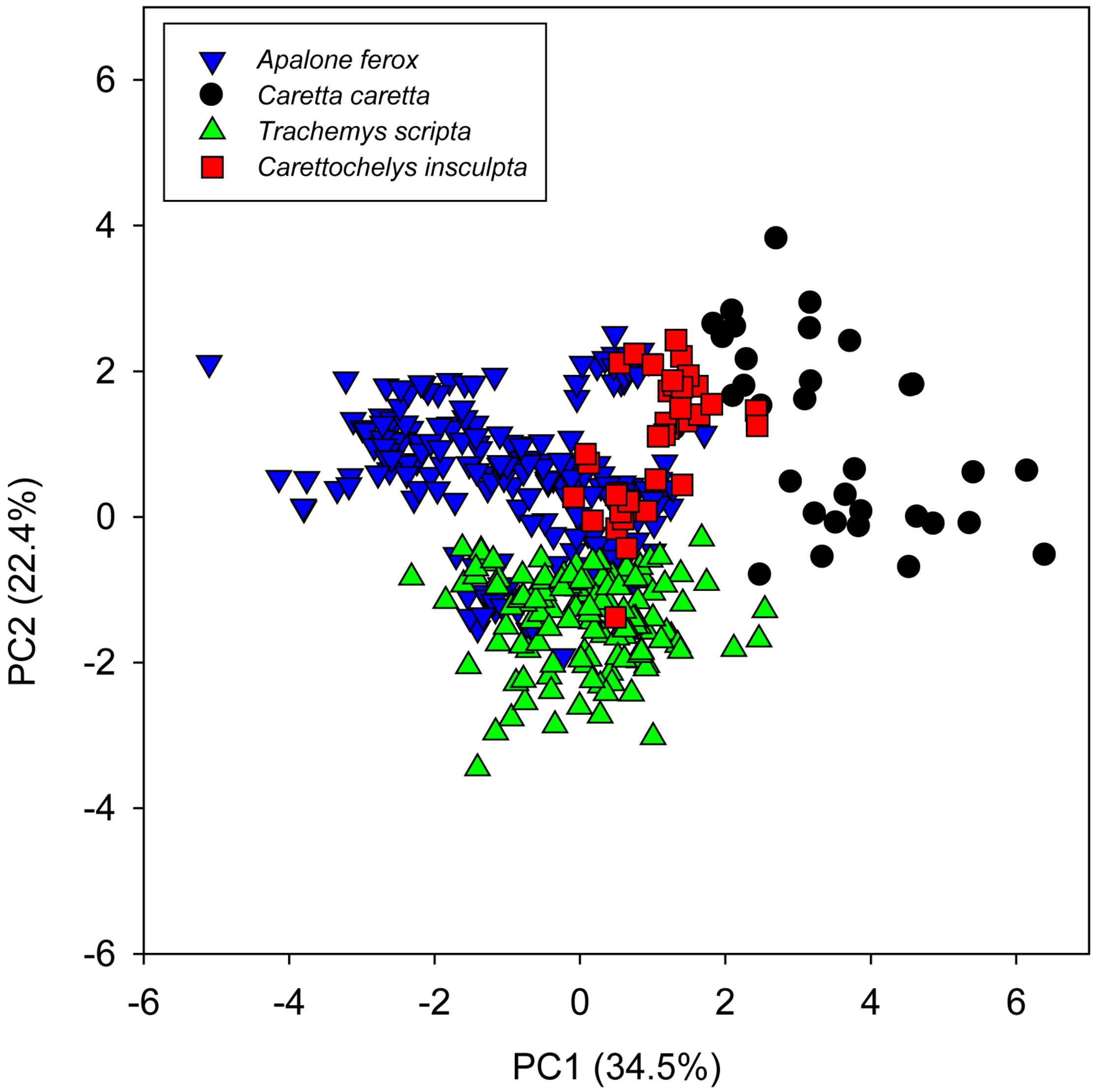

Fig. 4.3. Plot of the first two axes of a principle components analysis of swimming kinematics for eight variables in four species of turtle. The first two axes explain 56.9% of the total variation in forelimb swimming kinematics. Species included in this analysis are rowing *Apalone ferox* (blue inverted triangles), *Carettochelys insculpta* (red squares), rowing *Trachemys scripta* (green triangles), and flapping *Caretta caretta* (black circles).

Table 4.2. PC loadings from a principle component analysis of swimming kinematics for eight variables in four species of turtle

	PC1	PC2
Kinematic variables	34.5%	22.4%
Maximum humeral depression	-0.314	-0.454
Maximum humeral elevation	0.129	-0.662
Maximum humeral retraction	-0.321	0.404
Maximum humeral protraction	-0.411	-0.185
Maximum elbow flexion	0.443	0.276
Maximum elbow extension	0.472	-0.193
Maximum forefoot feathering	-0.369	-0.042
Minimum forefoot feathering	-0.243	0.207

Table 4.3. Euclidean distance matrix comparing kinematics of swimming in four species of turtle

	Apalone ferox	***Caretta caretta***	***Carettochelys insculpta***
Caretta caretta	4.56	----------	----------
Carettochelys insculpta	2.66	3.33	----------
Trachemys scripta	2.48	4.45	2.96

Based on standardized means (Z scores) for each species.

Calculated from 8 kinematic variables (maximum humeral depression, elevation, retraction, and protraction, maximum elbow flexion and extension, and maximum and minimum forefoot feathering). All pair-wise comparisons were significant ($P_{rand} < 0.001$).

Table 4.4. P-values from Tukey pair-wise mean comparisons of kinematic variables for four species of turtle

Variable	*A. ferox*	*T. scripta*	*C. insculpta*
Maximum humeral depression[1]			
T. scripta	0.831	-----	-----
C. insculpta	**0.010**	**0.004**	-----
C. caretta	**<0.001**	**<0.001**	**0.046**
Maximum humeral elevation[1]			
T. scripta	**0.005**	-----	-----
C. insculpta	0.995	0.064	-----
C. caretta	0.507	0.467	0.608
Dorsoventral humeral excursion angle[3]			
T. scripta	**<0.001**	-----	-----
C. insculpta	**0.003**	0.893	-----
C. caretta	**<0.001**	**<0.001**	**<0.001**
Maximum humeral retraction[1]			
T. scripta	**<0.001**	-----	-----
C. insculpta	**0.035**	0.293	-----
C. caretta	**0.007**	0.281	0.997
Maximum humeral protraction[1]			
T. scripta	0.998	-----	-----
C. insculpta	0.820	0.877	-----
C. caretta	**0.005**	**0.005**	**0.009**
% of limb cycle at maximum protraction[2]			
T. scripta	0.995	-----	-----
C. insculpta	**0.049**	**0.042**	-----
C. caretta	0.974	0.939	0.218
Anteroposterior humeral excursion angle[3]			
T. scripta	**<0.001**	-----	-----
C. insculpta	**0.005**	0.817	-----
C. caretta	0.688	**<0.001**	**0.003**

Table 4.4., continued

Maximum elbow flexion[1]			
T. scripta	0.925	-----	-----
C. insculpta	0.199	0.111	-----
C. caretta	0.119	0.060	1.000
Maximum elbow extension[1]			
T. scripta	0.069	-----	-----
C. insculpta	0.181	0.977	-----
C. caretta	**0.009**	0.384	0.808
% of limb cycle at maximum elbow extension[2]			
T. scripta	**0.027**	-----	-----
C. insculpta	0.585	**0.023**	-----
C. caretta	0.993	0.244	0.593
Elbow excursion angle[3]			
T. scripta	**0.018**	-----	-----
C. insculpta	0.986	0.107	-----
C. caretta	0.824	0.405	0.795
Maximum forefoot feathering[1]			
T. scripta	0.979	-----	-----
C. insculpta	0.605	0.480	-----
C. caretta	**0.007**	**0.005**	0.317
DV/AP excursion ratio of digit 3[4]			
T. scripta	0.918	-----	-----
C. insculpta	0.177	0.357	-----
C. caretta	**<0.001**	**<0.001**	**<0.001**

Tukey pair-wise mean comparison tests performed separately for each variable found significant in four-species tests (Table 4.1).

[1] Values are angles in degrees

[2] Values represent a percentage of the limb cycle

[3] Values represent the total angular excursion

[4] Ratio of dorsoventral (DV) to anteroposterior (AP) excursions of distal-most point of the forelimb (digit 3)

Significant pair-wise comparisons are shown in boldface.

4.1, 4.4). Additionally, flapping *C. caretta* protract the humerus significantly less than the three freshwater species (Fig. 4.2; Tables 4.1, 4.4). While the limb cycle was defined as protraction followed by retraction for the three freshwater species, for sea turtles (*C. caretta*) it was defined as humeral elevation (at 51±2.5% of the limb cycle) followed by depression (see Chapter 3). Despite this difference, all species exhibit humeral protraction during the first phase of the limb cycle (Fig. 4.2A), and only slight (though significant) differences were found in the timing of maximum protraction between *C. insculpta* (51±0.9%) and both *A. ferox* (43±0.6%) and *T. scripta* (43±0.6%) (Fig. 4.2A, Tables 4.1, 4.4). Similarly, the timing of maximum protraction in *C. caretta* (44±2.9%) did not differ from freshwater species.

Three distinct patterns of dorsoventral motion are seen among the four species (Fig. 4.2B). Rowing *T. scripta* and flapping *C. caretta* both are characterized by a single peak of elevation (coincident with the timing of peak protraction), while *C. insculpta* displays a bimodal pattern of humeral elevation, and *A. ferox* displays minimal humeral dorsoventral movement (Fig. 4.2B). Despite differences in the general pattern or presence of a peak in elevation, only minimal differences were found in the peak values of humeral elevation; *T. scripta* elevates the humerus significantly more than *A. ferox* (20±0.7 deg versus 2±0.7 deg), with values for *C. insculpta* (-1±2.2 deg) approaching a significant difference from *T. scripta* (*P* = 0.064; Fig. 4.2B, Tables 4.1, 4.4). Similarly, dorsoventral humeral excursion also exhibits three distinct patterns among the four species (Fig. 4.2B). *Apalone ferox* displays significantly less dorsoventral motion (13±0.4 deg) than other species, *C. insculpta* and *T. scripta* display ranges of motion similar to each other (31±1.4 deg and 28±0.7 deg) that are intermediate and significantly different than others, and finally, *C. caretta* displays the greatest range of dorsoventral motion (61±4.5 deg; $P < 0.001$ for all comparisons; Fig. 4.2B; Tables 4.1, 4.4). Maximum humeral depression was significantly greater in *C. caretta* (-51±2.6 deg) than in *C. insculpta* (-32±1.4 deg), and was significantly greater in *C. insculpta* and *C. caretta* than rowers, but rowing *A. ferox* (-11±0.6 deg) and *T. scripta* (-8±0.6 deg) did not differ (Fig. 4.2B; Tables 4.1, 4.4).

Motion at the elbow displays a generally similar pattern in all four species, extending during the first phase of the limb cycle with flexion beginning at roughly the same time as the second phase of the limb cycle (Fig. 4.2C). However, the pattern in *A. ferox* and *T. scripta* begins with a period of elbow flexion, reaching a similar maximum elbow flexion angle of 67±1.1 deg and 61±1.3 deg, respectively, at approximately 20% of the limb cycle (Fig. 4.2C; Tables 4.1, 4.4). *Carettochelys insculpta* and *C. caretta* begin and end each cycle with a maximally flexed elbow (92±1.3 deg and 93±3.6 deg; Fig. 4.2C; Tables 4.1, 4.4). While both rowers and species typically viewed as “flappers” display degrees of elbow flexion that are similar within these two categories, visibly different between categories, and were found to display significant differences via two-way ANOVA (Table 4.1), only the greatest difference, between rowing *T. scripta* and flapping *C. caretta*, approaches significance (*P* = 0.060; Table 4.4). Similarly, only minimal differences were found with regard to maximum elbow extension; only *A. ferox* and *C. caretta* differ (107±1.2 deg versus 139±3.1 deg; Fig. 4.2C; Tables 4.1, 4.4). Elbow excursion angle differs significantly only between *T. scripta* (62±1.5 deg) and *A. ferox* (40±1.0 deg), though *C. insculpta* displays the least motion at the elbow (36±1.1 deg); this discrepancy is likely due to the smaller sample size for the rare species, leading to a less powerful but more conservative statistical test. Finally, maximum elbow extension occurs significantly later in the limb cycle for *T. scripta* (68±1.3%) than for *A. ferox* (56±0.8) or *C. insculpta* (49±1.2%), but does not differ from that of *C. caretta* (59±4.0%; Fig. 4.2C; Tables 4.1, 4.4).

The four species display the fewest kinematic differences in forefoot feathering orientation (Fig. 4.2D), with only maximum (i.e., high-drag) forefoot orientation displaying significant differences (Table 4.1). All species display the same general pattern of rotating the forefoot (also called flipper in *C. insculpta* and *C. caretta*) into a maximally feathered (i.e., low-drag) orientation during the first phase of the limb cycle (“recovery phase”), followed by rotation to a high-drag orientation during the second phase of the limb cycle (“thrust phase”) (Fig. 4.2D). *Caretta caretta* is the only species to exhibit a negative inclination of the forefoot at any point of the swimming cycle (Fig. 4.2D). *Apalone ferox* and *Trachemys scripta* display significantly greater high-drag forefoot angles than *Caretta* (76±1.0 deg and 78±1.1 deg versus 54±3.1 deg), with *Carettochelys* also achieving higher, though not significantly different, values (67±1.9 deg) (Fig. 4.2D; Tables 4.1, 4.4).

Species also differed in regard to motion of the distal-most point of the forelimb (digit 3) (Fig. 4.4; Table 4.1). Despite greater dorsoventral motion in *T. scripta*, the trajectories of digit 3 for both A*. ferox* and *T. scripta* (asynchronous rowers) are horizontal and the ratios of dorsoventral to anteroposterior motion of digit 3 (DV/AP) in each does not differ significantly (*Apalone*$_{\mathrm{DV/AP}}$=0.23±0.01, *Trachemys*$_{\mathrm{DV/AP}}$=0.29±0.01; Fig. 4.4; Table 4.3). Flapping *C. caretta* approach (but do not attain) a vertical trajectory, with a DV/AP ratio that differs significantly from that of *A. ferox* and *T. scripta* (*Caretta*$_{\mathrm{DV/AP}}$=1.47±0.13; Fig. 4.4; Table 4.3). Finally, *C. insculpta* exhibit a trajectory of the tip of the flipper that is intermediate between *Apalone-Trachemys* and *Caretta* and a DV/AP ratio that differs significantly from that of *C. caretta* but that does not differ significantly from those of *A. ferox* and *T. scripta* (*Carettochelys*$_{\mathrm{DV/AP}}$=0.58±0.03; Fig. 4.4; Table 4.3).

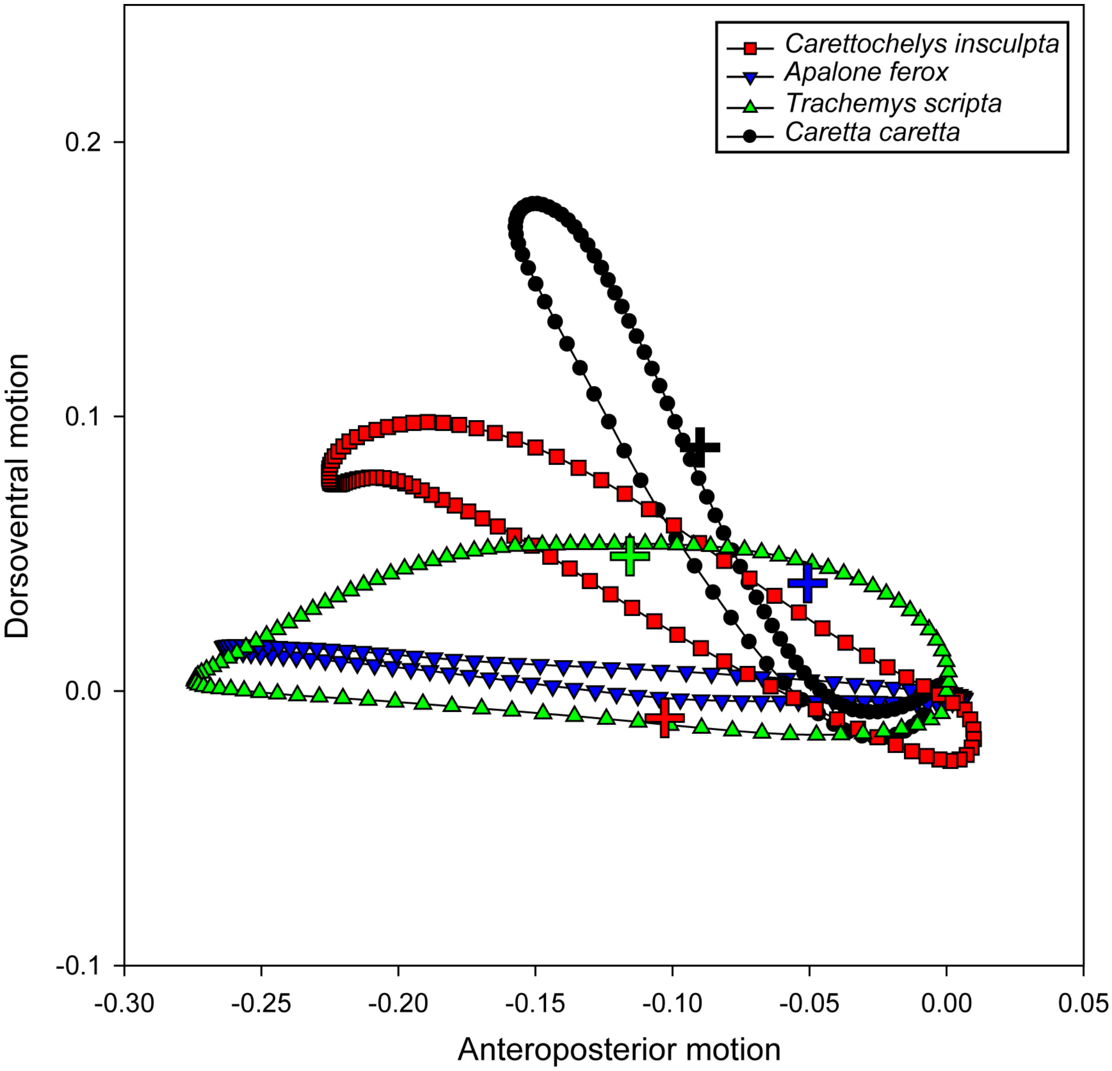

Fig. 4.4. Lateral view of the paths taken by the distal-most point of the forelimb (digit 3; tip of the flipper in *Carettochelys* and *Caretta*) for *Carettochelys insculpta* (red squares), *Apalone ferox* (blue inverted triangles), *Trachemys scripta* (green triangles), and *Caretta caretta* (black circles) showing the amount of anteroposterior and dorsoventral motion relative to the turtle's body throughout the limb cycle. Coordinate positions of X and Z throughout the swimming cycle were smoothed and interpolated to 101 points. Paths are the average of all trials for each species, and have been scaled to unit size to facilitate comparisons of trajectories. Paths start at the origin. Position of the shoulder relative to the path is indicated for each species with a color-coded cross. Despite greater dorsoventral motion in *T. scripta*, the trajectories of *A. ferox* and *T. scripta* (rowers) are both horizontal. *Caretta caretta* (flapper) approaches (but does not attain) a vertical trajectory. Finally, in *Carettochelys,* the trajectory of the tip of the flipper is intermediate between *Apalone-Trachemys* and *Caretta*. The ratios

of dorsoventral to anteroposterior motion of digit 3 designate *A. ferox*, *T. scripta*, and *C. insculpta* as rowers (ratios less than 1: $Apalone_{DV/AP}=0.23 \pm 0.01$, $Trachemys_{DV/AP}=0.29 \pm 0.01$, $Carettochelys_{DV/AP}=0.58 \pm 0.03$) and *C. caretta* as a flapper with a ratio greater than 1 ($Caretta_{DV/AP}=1.47 \pm 0.13$).

Discussion

Multivariate comparison of forelimb kinematics across swimming styles in turtles

Based on multivariate comparisons of kinematic parameters representative of the overall pattern of forelimb kinematics, I found significant differences among all of the species. Based on Euclidean distance analysis, the two freshwater species that use asynchronous rowing (*A. ferox* and *T. scripta*) were found to be most similar in forelimb kinematics ($D_{Apalone\text{-}Trachemys} = 2.48$; Fig. 4.3; Table 4.3). Between these rowers and the species typically considered "flappers", forelimb kinematics were most similar between the sister taxa *A. ferox* and *C. insculpta* ($D_{Apalone\text{-}Carettochelys} = 2.66$; Fig. 4.3; Table 4.3), followed by *T. scripta* and *C. insculpta* ($D_{Trachemys\text{-}Carettochelys} = 2.96$; Fig. 4.3; Table 4.3). The three largest pair-wise distances were between flapping sea turtles (*Caretta*) and the three freshwater species, but with *C. insculpta* being most similar to *Caretta* ($D_{Carettochelys\text{-}Caretta} = 3.33$; Fig. 4.3; Table 4.3). Finally, the rowing-style forelimb kinematics of swimming in *T. scripta* (a semi-aquatic generalist) were more similar to the flapping kinematics of *C. caretta* ($D_{Trachemys\text{-}Caretta} = 4.45$) than they were to those of the highly aquatic *A. ferox* ($D_{Apalone\text{-}Caretta} = 4.56$).

Comparison of rowing in Apalone ferox *and* Trachemys scripta

While rowing and flapping are really points along a continuum of possible limb motions, my data also support the conclusion that rowing should, itself, be viewed as a continuum. While forelimb kinematics are most similar between the two asynchronously rowing species, I found some strong differences between the kinematics employed by generalist rowers and specialist rowers. For example, *A. ferox* restricts the range of both anteroposterior and dorsoventral humeral motions by limiting humeral retraction and elevation compared to *T. scripta*. This is similar to the differences reported for *T. scripta* and another softshell species, *Apalone spinifera* (Pace et al., 2001). Rowing appears to be fairly similar between *A. ferox* and *A. spinifera*, though the latter primarily holds the humerus elevated with respect to the horizontal while the humerus of the former is generally depressed. In addition, when compared to *A. spinifera*, *A. ferox* displays a narrower range of anteroposterior motion [49 deg versus 74 deg (Pace et al., 2001)] and extends the elbow less [maximum elbow extension angle of 107 deg versus 149 deg (Pace et al., 2001)]. Although the limb cycle frequencies exhibited by each were similar (*A. ferox*=2.24±0.3 cycles/sec and *A. spinifera*=1.66±0.12 cycles/sec), it is possible that kinematic differences between *Apalone* species are due to differences in speed. Nevertheless, aquatic specialists may be more efficient swimmers due to the ways in which they limit extraneous humeral motions. However, whether the tendency to limit motion in aquatic specialists is an adaptation for increased swimming efficiency, or the greater range of motion exhibited by the semi-aquatic generalist *T. scripta* is related to the greater extent to which it moves over land, remains to be determined.

Comparison of swimming between Carettochelys insculpta *and other turtles*

Carettochelys and sea turtles are distantly related, yet have both arrived at a similar derived forelimb morphology (flippers) and synchronous mode of swimming through convergent evolution. Swimming in *Carettochelys insculpta* is typically described as flapping and being like that of sea turtles (Walther, 1921; Rayner, 1985; Georges et al., 2000), though formal comparisons of quantified kinematics had not been performed. My measurements indicate that *C. insculpta* and sea turtles have not converged on an identical flapping style of swimming through use of similar humeral kinematics. While both sea turtles (*C. caretta*) and *Carettochelys* swim via synchronous motions of the flippers, their movements are only superficially similar, as their patterns of humeral motion differ substantially. While the primary humeral motions in *C. caretta* are elevation and depression, this is not the case in *C. insculpta*, which shows a unique bimodal pattern of dorsoventral motion and does not depress the humerus nearly as much as *C. caretta. Carettochelys* also protract the humerus significantly more than *C. caretta* (and slightly more than the asynchronous rowers in my comparison), leading to a much greater anteroposterior range despite nearly identical levels of humeral retraction. Although these two species differ in the predominant directions of humeral motion (i.e., dorsoventral for *C. caretta* and anteroposterior for *C. insculpta*), they are quite similar with regard to motion at the elbow, indicating that this might be an aspect of kinematics important to producing dorsoventral motion of the flippers in both species.

Despite common statements to the contrary, I actually find the humeral kinematics of swimming in *Carettochelys* to be more similar to the rowing kinematics used by *A. ferox* and *T. scripta* than to the flapping kinematics of my sea turtle species (*C. caretta*); in fact, the multivariate analyses found the three freshwater species to be most similar. Humeral motion during the restricted rowing of *A. ferox* is more similar to that of *C. insculpta* than it is to the rowing of *T. scripta*. This similarity may reflect the close phylogenetic relationship between *A. ferox* and *C. insculpta.* Given the limited amount of humeral depression and retraction observed in *A. ferox* relative to *T. scripta*, it is clear why both dorsoventral and anteroposterior ranges of motion differ. *Trachemys scripta* also shows less humeral depression than *C. insculpta*, and while the pattern of anteroposterior movement is very similar between the two, *C. insculpta* reaches peak elbow extension significantly earlier. Patterns of forefoot feathering are nearly identical between *A. ferox* and *C. insculpta*, and with the exception of a mid-cycle high-drag peak, the pattern in *T. scripta* is also quite similar. While the humeral kinematics used by both asynchronous rowers were more similar to those of *C. insculpta*, and while *A. ferox* is most similar to *C. insculpta*, the biggest pair-wise species difference observed was between *A. ferox* and *C.*

caretta. *Apalone ferox* differs from *C. caretta* with regard to aspects of dorsoventral motion and maximum protraction, and in addition, retracts the humerus and extends the elbow significantly less. Differences between *T. scripta* and *C. caretta* are summarized in Chapter 3; briefly, *Trachemys* shows greater anteroposterior motion due to significantly greater protraction, while *Caretta* shows greater dorsoventral motion due to significantly greater humeral depression. *Apalone ferox* and *T. scripta* both achieve higher-drag forefoot orientations than observed in *C. caretta.*

How does Carettochelys insculpta swim?

My quantitative evaluation of forelimb kinematics during swimming in *C. insculpta* shows that this unusual freshwater species, which is commonly described as a flapper, displays limb motions that are similar to flappers for some parameters, but that more closely resemble the kinematics of rowers overall. So, how does *Carettochelys* swim? Humeral kinematics of swimming in *C. insculpta* are more similar to the rowing kinematics of *A. ferox* and *T. scripta*; they are not flapping the humerus up and down as seen for flapping *C. caretta*. *Carettochelys* shows a great amount of humeral protraction (slightly greater peak values than the rowers) and retraction, and a much smaller amount of elevation and depression than *C. caretta*. The key to how this species accomplishes what looks like flapping-style locomotion (and hence, the reason it has historically been described as a flapper) appears to lie in humeral rotation. As the humerus is protracted, the extent of elevation of the tips of the digits, while humeral elevation remains minimal, indicates substantial medial rotation while the elbow is extended. This rotation causes the flipper blade to elevate even as the distal end of the humerus starts to depress, resulting in what appears to be an upstroke of the limb and the first peak in humeral elevation. As the humerus is retracted it appears to rotate laterally while the elbow is flexed, causing the flipper blade to depress while the distal end of the humerus slightly elevates and results in an apparent downstroke of the limb and the second peak in elevation. *Carettochelys* reaches peak high-drag forefoot orientation concurrent with the slight second peak in humeral elevation, and then returns to the starting position. While the pattern of forefoot orientation in *C. insculpta* is very similar to that of the other freshwater species (both rowers), particularly *A. ferox*, rotation of the humerus in combination with a pattern of elbow motion that more closely resembles that of flapping *C. caretta* produces a pattern of limb motion in *C. insculpta* that bears a strong, though somewhat superficial, resemblance to movements typically viewed as "flapping". Thus, *Carettochelys and Caretta* show some components of convergence on what appears to be a flapping-style of swimming, though it is achieved with significant kinematic differences. While the pattern of motion at the elbow might play an important role in the generation of the upstroke and downstroke characteristic of flapping-style swimming, humeral elevation and depression appear to be crucial for generating flapping in

C. caretta while humeral rotation is more important in the generation of the "upstroke" and "downstroke" of *C. insculpta.*

Humeral motion does not support the classification of *C. insculpta* as a flapper. However, given the strong visual resemblance of the motions of *Carettochelys* limbs to flapping, might other kinematic variables indicate that *C. insculpta* swims via dorsoventral flapping, even though the most prominent humeral movements are not dorsoventral (i.e., upstroke and downstroke)? An additional way that species could be classified as flappers or rowers is by evaluating the amount of dorsoventral motion of the foot relative to anteroposterior motion; while equal amounts of dorsoventral and anteroposterior motion yield a ratio of 1, greater values indicate flapping, and smaller values are indicative of rowing. A comparison of the path traveled by the tip of the flipper (digit 3) shows that although *C. insculpta* exhibits far greater dorsoventral excursion than rowing *A. ferox* and *T. scripta*, there is still a greater amount of anteroposterior than dorsoventral motion in *C. insculpta* (Fig. 4.4). Based on the ratio of dorsoventral to anteroposterior motion of the distal-most tip of the forelimb, *A. ferox* and *T. scripta* are classified as rowers ($Apalone_{\mathrm{DV/AP}}=0.23\pm0.01$, $Trachemys_{\mathrm{DV/AP}}=0.29\pm0.01$), *C. caretta* as flappers ($Caretta_{\mathrm{DV/AP}}=1.47\pm0.13$), and *C. insculpta* as intermediate between these two groups ($Carettochelys_{\mathrm{DV/AP}}=0.58\pm0.03$), though still on the rowing side of this index. Thus, with forelimb kinematics showing aspects resembling both rowers and flappers, but more closely aligned with rowers based on multivariate results as well as overall flipper motion (i.e., DV/AP ratio less than 1), *C. insculpta* is perhaps best described as a rower (albeit with forelimbs moved synchronously). My classification of *C. insculpta* as a rower is further justified by the statistical findings (based on the ratio of dorsoventral to anteroposterior motion of digit 3) that indicated that *C. insculpta* is statistically different from flapping *Caretta*, but not from the traditionally classified rowers, *T. scripta* and *A. ferox*. Additionally, despite the convergence of some limb motions by *C. insculpta* on patterns like those of flapping sea turtles, among the species in my comparisons only the sea turtle *C. caretta* achieves a negative forefoot inclination during upstroke, suggesting the modulation of propulsor angle of attack typical for lift-based flight (Vogel, 1994). However, despite the evidence indicating that *C. insculpta* is best regarded as a synchronous rower, the extent to which the increased dorsoventral motion (i.e., DV/AP ratio) of *C. insculpta*, and even the true flapping of sea turtles, produces lift-based thrust remains to be tested (e.g., using digital particle image velocimetry).

Conclusions

I have shown that while *C. insculpta* does not show convergence with the flapping motions of sea turtles and rather is best described as a synchronous rower, that *C. insculpta* does exhibit a suite of swimming forelimb kinematics different from other species (e.g., substantial anteroposterior humeral motion, bimodal pattern of humeral elevation and depression, and an intermediate amount of distal dorsoventral limb motion). Interspecific variation in locomotor behaviors can arise through modification of anatomical structures, modification of patterns of muscle activation, or some combination of both. While I have identified these patterns of kinematic differences, a next step would involve determining how motor patterns are associated with generating these differences. A recent examination of the forelimb motor patterns that power swimming in *T. scripta* and *C. caretta* showed remarkable conservation in the activation patterns of several muscles (e.g., coracobrachialis, latissimus dorsi), but marked differences in others (e.g., deltoid, triceps), suggesting that the evolution of flapping in sea turtles was achieved through modification of structures (e.g., flippers) as well as motor patterns. Given the similarity of kinematics in *C. insculpta* to rowing in *A. ferox* and *T. scripta*, it is possible that *C. insculpta* might exhibit motor patterns more similar to those of other rowing freshwater species, particularly to those in the more closely related and more similar *Apalone* (*A. ferox* and *A. spinifera*). However, it remains to be seen how patterns of muscle activation compare among a broad range of rowing and flapping turtles. Testing this could give additional insight into how novel patterns of locomotion arise.

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CHAPTER FIVE

FORELIMB MUSCLE FUNCTION IN THE PIG-NOSED TURTLE, *CARETTOCHELYS INSCULPTA*: TESTING NEUROMOTOR CONSERVATION BETWEEN ROWING AND FLAPPING IN SWIMMING TURTLES

Abstract

Changes in muscle activation patterns can lead to new locomotor modes; however, neuromotor conservation has been documented across diverse styles of locomotion. Animals that swim using appendages do so by way of rowing or flapping. Yet, few studies have compared motor patterns between aquatic rowers and flappers. In swimming turtles, propulsion is generated exclusively by limbs. Kinematically, turtles swim using multiple styles of rowing (freshwater species), flapping (sea turtles), and a unique rowing style of swimming displaying superficial similarity to flapping in sea turtles and characterized by increased dorsoventral motions of synchronously oscillated forelimbs that have been modified into flippers (*Carettochelys insculpta*). I compared forelimb motor patterns in four species of turtle (two rowers, *Apalone ferox* and *Trachemys scripta*; one flapper, *Caretta caretta*; and *C. insculpta*) and found that despite kinematic differences, muscle activity patterns were generally similar among species with a few notable exceptions: specifically, the presence of variable bursts for pectoralis and triceps in *T. scripta* (though timing of the non-variable pectoralis burst was similar), and the timing of deltoideus activity in *C. insculpta* and *C. caretta* compared to other taxa. My data thus provide partial support for

neuromotor conservation among turtles using diverse locomotor styles, but implicate deltoideus activity as a prime contributor to flapping limb motions.

Introduction

The evolution of vertebrates has produced a variety of appendage-based locomotor modes (e.g., running, flying, and swimming) and associated morphologies. Among swimming taxa, vertebrate appendages have been modified for rowing or flapping. Though more accurately viewed as points along a continuum, rowing is characterized by anteroposterior oscillatory motions of paddle-shaped appendages, whereas flapping is characterized by dorsoventral oscillatory motions of wing-shaped appendages (Webb, 1984; Walker and Westneat, 2000). Rowing and flapping have been documented among diverse taxa, including fishes (Webb, 1984; Walker and Westneat, 2000; Walker, 2002; Walker and Westneat, 2002), turtles (Davenport et al., 1984; see Chapters 2 and 3), birds (Baudinette and Gill, 1985), and mammals (Feldkamp, 1987; Fish, 1996). Understanding how new locomotor modes arise, whether through changes in morphology, muscle activity, or a combination of both, is a major focus of evolutionary studies of musculoskeletal function. In particular, the idea that new behaviors can arise solely through changes in structure, without concurrent changes in the patterns of muscle activity that control movement of those structures, has been formalized as the ‘neuromotor conservation hypothesis’ (see Smith, 1994 for review). While evidence for neuromotor conservation is found across terrestrial and aerial locomotor modes (Jenkins and Goslow, 1983; Dial et al., 1991; Goslow et al., 2000), few studies have examined this for swimming, particularly between aquatic rowing and flapping.

Turtles represent an ideal group in which to study appendage-based locomotion because propulsive forces are generated exclusively by the limbs (Pace et al., 2001). Species of aquatic turtles swim via rowing or flapping: all but one freshwater species (over 200) swims using asynchronous rowing of paddle-shaped forelimbs and hindlimbs, whereas all marine turtles (seven species) swim using synchronous flapping of forelimbs that have been modified into flippers. A single freshwater species, *Carettochelys insculpta* (hereafter "*Carettochelys*"; Family Carettochelyidae), has converged on synchronous motions of foreflippers that superficially resemble flapping in sea turtles, but that are nevertheless best classified as rowing (albeit synchronously). While the dorsoventral component of forelimb motion in *Carettochelys* is increased relative to other freshwater rowers, the primary direction of forelimb movement is still anteroposterior, and overall kinematics resemble rowing in many respects (see Chapter 4). A recent examination of forelimb motor patterns in rowing *Trachemys scripta* (hereafter "*Trachemys*"; Family Emydidae) and flapping *Caretta caretta* (hereafter "*Caretta*"; Family Cheloniidae) showed remarkable conservation in the activation patterns of several muscles (e.g., coracobrachialis and latissimus dorsi), but marked differences in others (e.g., deltoideus and triceps), suggesting that the evolution of flapping in sea turtles (*Caretta*) was achieved through modification of structures (e.g., flippers), as well as motor patterns (see Chapter 3).

The primary goal of this study was to determine whether forelimb motor patterns during swimming in *Carettochelys* more closely resemble patterns of muscle activity in phylogenetically similar freshwater rowers or marine flappers, with whose locomotor style *Carettochelys* shares some similarities. To do this, I measured forelimb motor patterns in swimming *Carettochelys*, as well as *Apalone ferox* (hereafter "*Apalone*"; Family Trionychidae), a specialized rower and member of the sister taxon to the monotypic Carettochelyidae (Iverson et al., 2007; Barley et al., 2010), thus providing a phylogenetic comparison. I compare these results to previous measurements from a generalized rower (*Trachemys*) and a flapping sea turtle (*Caretta*). Finally, by comparing motor patterns from 4 of the 13 families containing aquatic species, I was able to test for neuromotor conservation across a broad range of taxa using a variety of locomotor modes, including generalized and specialized rowing [which differ in aspects of kinematics (see Chapter 4)], flapping, and a unique style of rowing locomotion that displays similarities with both typical freshwater rowers and marine flappers.

Materials and Methods

Animals

Access to turtles was provided by a commercial vendor (Turtles and Tortoises Inc., Brooksville, FL, USA). Data were collected from two pig-nosed turtles, *Carettochelys insculpta* (carapace length = 23.8±1.8 cm), and nine Florida softshell turtles, *Apalone ferox* (carapace length = 15.1±1.1 cm). Turtles were housed in stock tanks (see Chapter 4 for details). The number of *Carettochelys* was limited due to their infrequent availability.

Collection and analysis of electromyography (EMG) data

Bipolar stainless steel electrodes (0.05 mm diameter, California Fine Wire Co., USA) were implanted percutaneously into target muscles of the left forelimb of *Carettochelys* (see Chapter 3 for details) and *Apalone* (see Chapter 2 for details) to generate data for comparison to other species. Protocols differed only slightly for the species; in particular, *Carettochelys* received local anesthetic (lidocaine) at implant sites and was tested the same day, whereas *Apalone* was anesthetized with ketamine prior to implants and tested the following day. EMG data were synchronized with kinematics (detailed in Chapter 4) and analyzed in LabVIEW.

I focused on five target muscles, covering all major planes of motion of the forelimb during swimming. Predicted actions were based on anatomical position: coracobrachialis (humeral retraction; not collected for *Carettochelys*), pectoralis (humeral retraction and depression), latissimus dorsi and deltoideus (humeral protraction and elevation), and the triceps complex (elbow extension) (Walker, 1973; Wyneken, 2001).

Statistical analysis

To assess general patterns of muscle function for each species, means and standard errors for each variable were calculated across all swimming trials (Table 5.1; see Appendix G). Muscle activity variables include, for each muscle: (i) onset, (ii) offset, and (iii) duration. Data for *Carettochelys* and *Apalone* were compared to those previously published for *Trachemys* (see Chapter 2) and *Caretta* (see Chapter 3) to assess how motor patterns during swimming compare among the species. Interspecific differences ($P<0.05$) were tested for using separate two-factor nested ANOVAs (SYSTAT), with individual nested within species, followed by Tukey post-hoc tests to evaluate each pair-wise species comparison.

Results

Forelimb motor patterns for *Carettochelys* and *Apalone* were calculated and compared to those published for *Caretta* and *Trachemys* (Fig. 5.1; Table 5.1). Patterns are very similar across species for humeral retractors. There are statistically significant, but minor, differences in onset of coracobrachialis (*Caretta* later than *Apalone*), and offset of pectoralis (*Caretta* earlier than *Trachemys* and *Carettochelys*). *Trachemys* also exhibits a variable early burst of activity in pectoralis. Among humeral protractors, the pattern of activation for latissimus dorsi is remarkably similar, with only a minor difference in offset between *Apalone* and *Caretta.* However, the pattern for deltoideus shows

Table 5.1. Mean values and standard errors for EMG timing variables, F-values for the main effect of species, and Tukey pair-wise mean comparison results

Variable	*Trachemys scripta*	*Apalone ferox*	*Carettochelys insculpta*	*Caretta caretta*	*F*-value	d.f.	Tukey Results
Coracobrachialis							
Onset	51±1.0	42±0.8	No data	62±1.3	5.16*	2,9	AF-CC
Offset	85±0.5	83±0.4	No data	84±1.3	0.83	2,9	
Duration	34.9±1.2	42±1.1	No data	21±1.4	3.01	2,9	
Pectoralis[1]							
Onset	62±1.5	51±0.6	57±0.9	57±1.7	1.19	3,11	
Offset	89±0.7	84±0.4	91±1.1	78±1.1	5.30*	3,11	CC-CI; CC-TS
Duration	28±1.3	33±0.7	34±1.1	21±1.3	1.24	3,11	
Latissimus dorsi[2]							
Onset	83±1.0	88±0.8	94±1.1	91±0.9	1.60	3,7	
Offset	35±0.9	27±1.5	37±1.4	39±1.2	4.36*	3,7	AF-CC
"Burst 1" Duration	35±0.9	27±1.5	37±1.4	37±1.2	3.70	3,7	
"Burst 2" Duration	16±1.0	12±0.8	6±1.1	8±0.9	1.67	3,7	
Total Duration	51±1.3	36±2.5	41±2.0	44±1.6	1.89	3,7	
Deltoid[3]							
Onset	96±0.4	94±0.3	81±2.2	60±1.3	217.42***	3,10	All but AF-TS[†]
Offset	33±0.9	30±0.8	7±1.2	84±1.0	39.59***	3,12	All but AF-TS[†]
				[-16±1.0]			
"Burst 1" Duration	30±1.1	30±0.8	7±1.2	-----	6.75*	2,10	AF-CI; CI-TS
"Burst 2" Duration	4±0.4	6±0.3	18±2.2	-----	26.94***	2,8	AF-CI; CI-TS
Total Duration	32±1.7	35±0.8	20±2.3	24±1.4	2.27	3,12	
Triceps[4]							
Onset	23±1.3	94±0.4	89±1.5	90±0.8	11.09**	3,11	AF-TS; CC-TS; CI-TS
	[123±1.3]						
Offset	51±1.5	39±0.6	38±1.4	44±1.5	1.61	3,12	
"Burst 1" Duration	-----	37±0.7	38±1.4	40±1.4	0.53	2,8	
"Burst 2" Duration	-----	6±0.4	11±1.5	10±0.8	1.93	2,7	
Total Duration	28±0.9	41±0.8	47±2.5	45±1.8	5.13*	3,12	CC-TS; CI-TS

Table 5.1, continued

"Burst #1" and "Burst #2" indicate early and late phase muscle activity of continuous muscles.

[1] *Apalone, Carettochelys,* and *Caretta* exhibit one discrete burst which was compared to the presumptive homologous non-variable later burst in *Trachemys*.

[2] Latissimus dorsi shows a continuous burst that spans the retraction/depression to protraction/elevation phase shift.

[3] *Apalone, Carettochelys,* and *Trachemys* each show one continuous burst (onset=start "Burst 2", offset=end "Burst 1") that was compared to the single discrete burst in *Caretta* (offset=[transposed value, by subtracting 100]).

[4] *Apalone, Carettochelys, and Caretta* exhibit one continuous burst (onset=start "Burst 2", offset=end "Burst 1") that was compared to the discrete non-variable early burst observed in *Trachemys* (onset=[transposed value, by adding 100]).

Values are means ± s.e.m.

$^{*}P\leq0.05$; $^{**}P\leq0.01$; $^{***}P\leq0.001$

Significant differences in pair-wise comparisons are indicated. AF=*Apalone ferox*; CC=*Caretta caretta*; CI=*Carettochelys insculpta*; TS=*Trachemys scripta*

[†] Indicates that all pair-wise comparisons, except for AF-TS, were significantly different

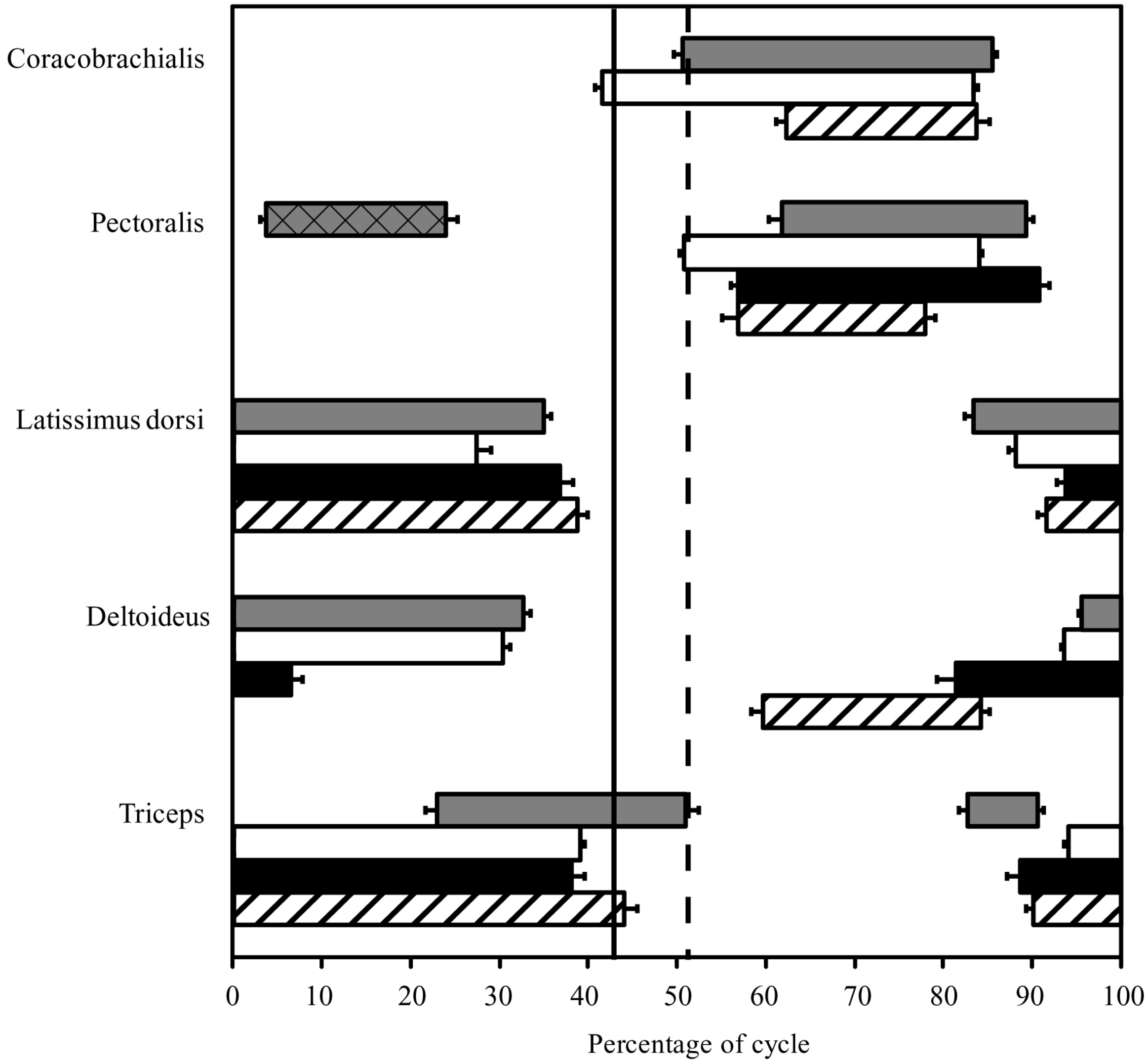

Figure 5.1. Bar plot showing mean (± s.e.m.) pattern of forelimb muscle activation during swimming in *Trachemys scripta* (gray, hatched=variable), *Apalone ferox* (white), *Carettochelys insculpta* (black), and *Caretta caretta* (diagonals). Vertical lines demarcate switch from protraction/elevation to retraction/depression (solid=*Trachemys/Apalone*, dashed=*Carettochelys/Caretta*).

marked differences among the species. Flapping *Caretta* exhibit a single discrete burst of deltoideus activity during humeral depression and retraction [opposite of the predicted action (Walker, 1973; see Chapter 3)], differing significantly in onset and offset from the three freshwater species. Furthermore, deltoideus activity in *Apalone* and *Trachemys* starts and ends significantly later than in *Carettochelys*, resulting in significantly increased duration of activity during protraction in the former. Finally, triceps differs primarily among the species in that *T. scripta* exhibits two bursts of activity (the second being variable and not always present). Onset of the primary triceps burst occurs significantly later in *Trachemys*, and while offset did not differ, duration in *Trachemys* was significantly shorter than in *Caretta* and *Carettochelys*.

Discussion

Muscles were active during the predicted portions of the limb cycle and showed similar patterns among species with few exceptions, including two instances of variable bursts in *Trachemys* and, most notably, activity of the deltoideus in *Caretta* (Fig. 5.1).

Trachemys exhibits a variable early burst for pectoralis not seen in other species; additionally, *Trachemys* exhibits two discrete bursts of triceps activity (the late burst being variable) whereas other species show a single continuous burst spanning the retraction/depression to protraction/elevation phase shift.

Of particular interest, my new results reveal that some differences in motor patterns (i.e., presence or absence of variable bursts) between *Trachemys* and *Caretta* observed in Chapter 3 are not attributable to their difference in swimming mode (i.e., rowing versus flapping), as was previously proposed, because rowing *Apalone* also lack variable pectoralis and triceps bursts, as well as differing in timing of triceps onset from *Trachemys*. Among rowers, timing of peak elbow extension occurs significantly later in *Trachemys*; additionally, the arm is held straighter near the end of the limb cycle (see Chapter 4). Thus, differences in triceps between rowers are associated primarily with differing elbow kinematics. Furthermore, the absence of a variable late triceps burst in the highly aquatic *Apalone*, *Caretta,* and *Carettochelys* versus its presence in semi-aquatic *Trachemys* might be a constraint on motor pattern associated with needing to move effectively over land, as walking motor patterns in *Trachemys* exhibit two bursts (see Chapter 2).

The primary difference in motor patterns among species occurs in the deltoideus. Chapter 3 concludes that the functional role of the deltoideus in *Caretta* has shifted during the evolution of flapping in turtles to serve as a stabilizer, minimizing anteroposterior humeral movements during the downstroke through simultaneous activation with pectoralis. The timing (though not duration) of deltoideus activity in *Carettochelys* differs from that of flapping *Caretta*, as well as rowing *Apalone* and *Trachemys.* Additionally, in comparison to asynchronous freshwater rowers, the duration of deltoideus activity in *Carettochelys* is significantly longer during retraction/depression and shorter during protraction/elevation. Thus, the pattern of deltoideus activity associated with the uniquely synchronous rowing by *Carettochelys* is intermediate between rowing and flapping, showing a shift towards the pattern observed in *Caretta*.

In conclusion, my data show a general trend of conservation of motor pattern among swimming turtles using a variety of locomotor styles, ranging from generalized and specialized rowing to flapping, and including the unique synchronous rowing of *Carettochelys*. Some variable muscle activity patterns found in more terrestrial *Trachemys* were absent from highly aquatic species, suggesting that the degree of terrestriality might impose certain constraints on motor pattern. Additionally, the deltoideus shows an evolutionary shift in timing that is drastic in flapping *Caretta* and intermediate in *Carettochelys.* Thus, this study provides partial support for the hypothesis of neuromotor conservation, with some muscles showing interspecific similarity, but others showing differences. These results suggest that evolutionary changes in muscle activation may occur more readily for some muscles (e.g., deltoideus) while illustrating the ability of evolution to produce completely new forms of locomotion through simple shifts in activation timing of a single muscle.

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APPENDICES

Appendix A

Sample Sizes of Aquatic and Terrestrial Locomotor Cycles Used for Kinematic

Turtle	Aquatic Cycles	Terrestrial Cycles
TS02	16	17
TS09	20	20
TS11	20	18
TS14	20	16
TS31	20	26
TS36	20	4
TS99	20	22

Appendix B

Aquatic and Terrestrial Sample Sizes for Each Turtle for EMG Variables for Statistics

Variable	Turtles (Aquatic Cycles, Terrestrial Cycles)
Coracobrachialis	
All EMG Timing Variables	TS09 (20, 0); TS11 (20, 0); TS14 (20, 0); TS31 (0, 24); TS36 (20, 4)
Normalized Amplitude	TS36 (20, 4)
Pectoralis Burst #1*	
All EMG Variables	TS09 (20, 20); TS11 (18, 18); TS99 (2, 17)
Pectoralis Burst #2*	
All EMG Variables	TS02 (16, 17); TS09 (20, 20); TS11 (20, 18); TS31 (20, 26); TS99 (20, 17)
Latissimus dorsi "Burst #1"	
All EMG Variables	TS11 (20, 18); TS31 (20, 24); TS36 (20, 4)
Latissimus dorsi "Burst #2"	
All EMG Variables	TS11 (20, 17); TS31 (20, 21); TS36 (20, 4)
Deltoid "Burst #1"	
All EMG Variables	TS09 (20, 20); TS11 (9, 5); TS14 (20, 5); TS31 (20, 26); TS99 (20, 21)
Deltoid "Burst #2"	
All EMG Variables	TS09 (7, 9); TS14 (14, 3); TS31 (1, 12); TS99 (20, 20)
Triceps Burst #1	
All EMG Timing Variables	TS02 (16, 17); TS11 (20, 8); TS14 (20, 16); TS31 (20, 10); TS99 (20, 22)
Normalized Amplitude	TS11 (20, 8); TS14 (20, 16); TS31 (5, 10); TS99 (20, 22)
Triceps Burst #2	
All EMG Timing Variables	TS02 (16, 17); TS11 (11, 8); TS14 (10, 16); TS31 (15, 9); TS99 (20, 22)
Normalized Amplitude	TS11 (11, 8); TS14 (10, 16); TS31 (4, 9); TS99 (20, 22)

Appendix B, continued

Variable	Turtles (Aquatic Cycles, Terrestrial Cycles)
Subscapularis (lat approach)	
All EMG Variables	TS11 (20, 17)
Subscapularis (cor approach)	
All EMG Variables	TS11 (20, 18); TS14 (20, 16)
Supracoracoideus (ant head)	
All EMG Variables	TS14 (18, 12)

All EMG Timing Variables = Onset, Relative Onset, Offset, Relative Offset, and Duration

All EMG Variables = All EMG Timing Variables and Normalized Amplitude

Burst #1 and "Burst #2" are used to indicate early and late activity, respectively, of a muscle exhibiting continuous activity that spans the retraction to protraction phase shift. These muscles include deltoid and latissimus dorsi.

* Aquatic EMGs for pectoralis showed early and late bursts of activity, but terrestrial EMGs showed only a single, late burst. Because the pectoralis burst common to both habitats was later in the limb cycle, it is coded as Burst #2 even if there was only a single burst. Because terrestrial EMGs only showed a single burst, statistical comparisons were run in two ways: Aquatic Burst #1 vs Terrestrial Burst and Aquatic Burst #2 vs Terrestrial Burst.

Appendix C

Aquatic and Terrestrial Sample Sizes for Each Turtle for Each Plotted EMG Timing Variable

Variable	Turtles (Aquatic Cycles, Terrestrial Cycles)
Coracobrachialis	TS09 (20, 0); TS11 (20, 0); TS14 (20, 0); TS31 (0, 24); TS36 (20, 4)
Pectoralis Burst #1*	TS02 (0, 17); TS09 (20, 20); TS11 (18, 18); TS31 (0, 26); TS99 (2, 17)
Pectoralis Burst #2*	TS02 (16, 0); TS09 (20, 0); TS11 (20, 0); TS31 (20, 0); TS99 (20, 0)
Latissimus dorsi "Burst #1"	TS11 (20, 18); TS31 (20, 24); TS36 (20, 4)
Latissimus dorsi "Burst #2"	TS11 (20, 17); TS31 (20, 21); TS36 (20, 4)
Deltoid "Burst #1"	TS09 (20, 20); TS11 (9, 5); TS14 (20, 5); TS31 (20, 26); TS36 (20, 0); TS99 (20, 21)
Deltoid "Burst #2"	TS09 (7, 9); TS14 (14, 3); TS31 (1, 12); TS99 (20, 20)
Triceps Burst #1	TS02 (16, 17); TS09 (0, 19); TS11 (20, 8); TS14 (20, 16); TS31 (20, 10); TS99 (20, 22)
Triceps Burst #2	TS02 (16, 17); TS09 (0, 19); TS11 (11, 8); TS14 (10, 16); TS31 (15, 9); TS99 (20, 22)
Subscapularis (lat approach)	TS11 (20, 17)
Subscapularis (cor approach)	TS11 (20, 18); TS14 (20, 16)
Supracoracoideus (ant head)	TS11 (3, 0); TS14 (18, 12)
Supracoracoideus (post head)	TS02 (16, 0)

cor approach = the electrode was implanted into the muscle by inserting it more posterior and laterally (as if approaching coracobrachialis); lat approach = the electrode was implanted into the muscle by inserting it more anteriorly (as if approaching latissimus dorsi); ant = anterior; post = posterior

"Burst #1" and "Burst #2" refer to the early and late, respectively, bursts of activity seen in muscles that present as a single continuous burst of activity that spans the switch from retraction to protraction.

* Aquatic EMGs for pectoralis showed a variable early burst that has been coded as Burst #1, with the "typical" burst of activity being coded always as Burst #2, even if there is only a single burst. In this case, 1 and 2 refer to "early" and "late" activity. Terrestrial EMGs only showed a single burst of activity that is always coded above as Burst #1.

Appendix C lists all cycles used to construct plots of EMG activity, whereas Appendix B only lists those cycles used in statistical analyses (ones in which the same electrode was active during swimming and walking).

Appendix D

Number of Swimming Trials for Kinematic Analyses for Each Turtle from Each Species

Caretta caretta		
	Log04	11
	Log05	8
	Log07	14
Trachemys scripta		
	TS02	16
	TS09	20
	TS11	20
	TS14	20
	TS31	20
	TS36	20
	TS99	20

Appendix E

Sample Sizes for EMG Timing Variables for Each Individual of Both Species for Plots and Statistics

Variable	*Caretta caretta*	*Trachemys scripta*
Coracobrachialis	Log04 (11); Log07 (6)	TS09 (20); TS11 (20); TS14 (20); TS36 (20)
Pectoralis Burst #1*	Log05 (8); Log07 (14)	TS09 (20); TS11 (18); TS99 (2)
Pectoralis Burst #2*	--------------------------------------	TS02 (16); TS09 (20); TS11 (20); TS31 (20); TS99 (20)
Latissimus dorsi "Burst #1"	Log04 (11); Log05 (8); Log06 (12); Log07 (8)	TS11 (20); TS31 (20); TS36 (20)
Latissimus dorsi "Burst #2"	Log04 (10); Log05 (8); Log06 (10); Log07 (5)	TS11 (20); TS31 (20); TS36 (20)
Deltoideus Burst #1**	Log04 (11); Log05 (6); Log07 (14)	--------------------------------------
Deltoideus “Burst #1”**	--------------------------------------	TS09 (20); TS11 (9); TS14 (20); TS31 (20); TS36 (20); TS99 (20)
Deltoideus “Burst #2”**	--------------------------------------	TS09 (7); TS14 (14); TS31 (1); TS99 (20)
Triceps “Burst #1”***	Log04 (11); Log05 (8); Log07 (14)	--------------------------------------
Triceps “Burst #2”***	Log04 (5); Log07 (14)	--------------------------------------
Triceps Burst #1***	--------------------------------------	TS02 (16); TS11 (20); TS14 (20); TS31 (20); TS99 (20)
Triceps Burst #2***	--------------------------------------	TS02 (16); TS11 (11); TS14 (10); TS31 (15); TS99 (20)

Sample sizes for each turtle are listed parenthetically.

"Burst #1" and "Burst #2" refer to the early and late, respectively, bursts of activity seen in muscles that present as a single continuous burst of activity that spans the switch from retraction to protraction.

* *Caretta* only exhibits one burst of EMG activity for pectoralis; it is coded as Burst #1. In contrast, *Trachemys* showed a variable early burst for pectoralis that has been coded as Burst #1, with the "typical" burst of activity being coded always as Burst #2, even if there is only a single burst. In this case, 1 and 2 refer to "early" and "late" activity.

** *Caretta* only exhibits one burst of EMG activity for deltoideus; it is coded as Burst #1. In contrast, *Trachemys* showed a single continuous burst of activity spanning the switch from retraction to protraction, referred to as "Burst #1" and "Burst #2".

*** *Caretta* exhibits a single continuous burst of triceps EMG activity that spans the switch from depression to elevation; these periods of activity are referred to as "Burst #1" and "Burst #2", respectively indicating the early and late periods of activity. *Trachemys* exhibits two separate and distinct bursts of activity referred to as Burst #1 and Burst #2.

Appendix F

Sample Sizes for Kinematic Analyses for Each Turtle from Each Species

Apalone ferox		
	AF01	20
	AF02	20
	AF03	25
	AF04	22
	AF05	20
	AF06	24
	AF07	20
	AF08	22
	AF09	22
Carettochelys insculpta		
	Flipper	22
	Chiquita	17
Caretta caretta		
	Log04	11
	Log05	8
	Log07	14
Trachemys scripta		
	TS02	16
	TS09	20
	TS11	20
	TS14	20
	TS31	20
	TS36	20
	TS99	20

Data for *Trachemys scripta* provided in Chapter 2. Data for *Caretta caretta* provided in Chapter 3.

Appendix G

Sample Sizes for Each Species for Each Turtle for EMG Timing Variables for Plots and Statistics

Muscle	*T. scripta*	*A. ferox*	*C. insculpta*	*C. caretta*
Coracobrachialis	TS09 (20) TS11 (20) TS14 (20) TS36 (20)	AF02 (20) AF03 (25) AF04 (22) AF06 (25) AF07 (20) AF08 (22)	No data	Log04 (11) Log07 (6)
Pectoralis (Variable early burst)	TS09 (20) TS11 (18) TS99 (2)	Not present	Not present	Not present
Pectoralis	TS02 (16) TS09 (20) TS11 (20) TS31 (20) TS99 (20)	AF01 (20) AF02 (20) AF03 (25) AF04 (22) AF05 (20) AF06 (25)	Chiquita (13) Flipper (22)	Log05 (8) Log07 (14)
Latissimus dorsi "Burst #1"	TS11 (20) TS31 (20) TS36 (20)	AF03 (4) AF06 (25)	Chiquita (15) Flipper (8)	Log04 (11) Log05 (8) Log06 (12) Log07 (8)
Latissimus dorsi "Burst #2"	TS11 (20) TS31 (20) TS36 (20)	AF03 (6) AF06 (25)	Chiquita (16) Flipper (7)	Log04 (10) Log05 (8) Log06 (10) Log07 (5)
Deltoideus "Burst #1"	TS09 (20) TS11 (9) TS14 (20) TS31 (20) TS36 (20) TS99 (20)	AF03 (25) AF04 (22) AF05 (20) AF08 (22) AF09 (22)	Chiquita (4) Flipper (10)	*C. caretta* displays a single discrete burst; listed below as Burst #1

Appendix G, continued

Muscle	*T. scripta*	*A. ferox*	*C. insculpta*	*C. caretta*
Deltoideus "Burst #2"	TS09 (7) TS14 (14) TS31 (1) TS99 (20)	AF03 (22) AF04 (20) AF05 (14) AF08 (9) AF09 (22)	Chiquita (8) Flipper (12)	
Deltoideus Burst #1	Each displays a single continuous burst which spans switch from retraction to protraction; "Burst #1" and "Burst #2" above indicate early and late activity			Log04 (11) Log05 (6) Log07 (14)
Triceps "Burst #1"	*T. scripta* displays two discrete bursts, the later being variable; listed below as Burst #1 and Burst #2	AF01 (9) AF03 (25) AF05 (9) AF06 (25) AF07 (20) AF09 (22)	Chiquita (17) Flipper (22)	Log04 (11) Log05 (8) Log07 (14)
Triceps "Burst #2"		AF01 (4) AF03 (25) AF05 (6) AF06 (20) AF07 (7) AF09 (9)	Chiquita (12) Flipper (20)	Log04 (5) Log07 (14)
Triceps Burst #1	TS02 (16) TS11 (20) TS14 (20) TS31 (20) TS99 (20)	Each displays a single continuous burst which spans switch from retraction to protraction; "Burst #1" and "Burst #2" above indicate early and late activity		
Triceps Burst #2 (Variable late burst)	TS02 (16) TS11 (11) TS14 (10) TS31 (15) TS99 (20)	Not present	Not present	Not present