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## RESEARCH ARTICLE

# Forelimb kinematics and motor patterns of swimming loggerhead sea turtles (*Caretta caretta*): are motor patterns conserved in the evolution of new locomotor strategies?

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### SUMMARY

Novel functions in animals may evolve through changes in morphology, muscle activity or a combination of both. The idea that new functions or behavior 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 evolutionary transitions in the behavior of terrestrial species, and in evolutionary transitions from terrestrial species to flying species. However, evolutionary transitions in the locomotion of aquatic species have received little comparable study to determine whether changes in morphology and muscle function were coordinated through the evolution of new locomotor behavior. To evaluate the potential for neuromotor conservation in an ancient aquatic system, we 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. We compared kinematic and motor patterns from loggerheads with previous data from the red-eared slider, *Trachemys scripta*, a generalized freshwater species exhibiting unspecialized forelimb morphology and anteroposterior rowing motions during swimming. For some forelimb muscles, comparisons between *C. caretta* and *T. scripta* support neuromotor conservation; for example, the coracobrachialis and the latissimus dorsi show similar activation patterns. However, other muscles (deltoideus, pectoralis and triceps) do not show neuromotor conservation; for example, the deltoideus changes 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.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/214/19/3314/DC1>

Key words: biomechanics, electromyography, kinematics, locomotion, muscle, neuromotor conservation.

### 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 (Smith, 1994). 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 (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). 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 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, such as 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). Although 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 (Zug, 1971; Renous et al., 2008) 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 hindlimbs) 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 turtle employ this mode of swimming, which is facilitated by derived modification of the forelimbs into elongate, semi-rigid flippers.

Novel behavior, including patterns of limb motion such as aquatic flapping, might arise through modification of structures, 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 whereas 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 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 this study, we tested whether differences in muscle function correlated with changes in limb morphology and locomotor behavior in turtles. We quantified forelimb motor patterns exhibited during flapping-style swimming by the loggerhead sea turtle, *Caretta caretta* (Linnaeus 1758), and compared these motor patterns with those recently published for the rowing-style swimming of a generalized freshwater turtle, the red-eared slider, *Trachemys scripta* (Schoepff 1792) (Rivera and Blob, 2010). *Trachemys scripta* is a member of the emydid lineage and, as such, is not a member of the sister clade to sea turtles (Barley et al., 2010). Although our comparison involves only two species and, thus, must be viewed cautiously in an evolutionary context (Garland and Adolph, 1994), because *T. scripta* is generally similar to the majority of freshwater turtles in limb morphology and swimming style (Zug, 1971; Walker, 1973; Pace et al., 2001; Blob et al., 2008; Barley et al., 2010), it is a reasonable model of basal states in turtle swimming for comparison with the derived flapping behavior of *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. In particular, given that at least moderate elevation and depression of the forelimbs is seen in sliders during rowing (Pace et al., 2001; Rivera and Blob, 2010), but that humeral protraction and retraction are generally restricted in flapping sea turtles (Wyneken, 1997), we would predict neuromotor conservation to be more likely among humeral elevators and depressors than among humeral protractors and retractors.

## MATERIALS AND METHODS

### Experimental animals

Data were collected from four juvenile (pelagic stage) loggerhead sea turtles that were similar in straight carapace length (59–65 mm; mean  $\pm$  s.e.m. =  $62 \pm 1.0$  mm) and body mass (31.7–45.8 g; mean  $\pm$  s.e.m. =  $41.4 \pm 1.3$  g). 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  $20 \times 20 \times 20$  cm plastic mesh baskets, which were placed into large tanks equipped with flow-through filtered seawater maintained at  $27 \pm 2^\circ\text{C}$  (the approximate thermal conditions in the Gulf Stream). A 12h:12h light:dark photoperiod was maintained with natural spectrum fluorescent lighting. Turtles were fed once daily using an in-house manufactured diet [detailed in Stokes et al. (Stokes et al., 2006); for further details on housing conditions and diet, see Dougherty et al. (Dougherty et al., 2010)]. Studies were conducted at Florida Atlantic University in accordance with IACUC guidelines (protocol 07-17, Florida FWC Turtle Permit no. 073, and USFWS permit TE056217-2). Experimental procedures followed those of our previous study of slider turtles (Rivera and Blob, 2010) as closely as possible to facilitate comparisons of data between these species, although the sea turtles that were available were somewhat smaller in size than the juvenile sliders from which data had been collected ( $145 \pm 6.0$  mm straight carapace length,  $450 \pm 42$  g).

### Collection and analysis of kinematic data

Locomotor trials, which consisted of limb cycles selected from the middle of a series of consecutive strokes (see supplementary material Table S1), were conducted in a custom-built aquarium with transparent glass sides and bottom ( $76 \times 32 \times 30$  cm length  $\times$  width  $\times$  height;  $\sim 1200$  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 deg angle to the transparent bottom of the tank. Turtles were filmed swimming fully submerged in still water at  $27 \pm 2^\circ\text{C}$ . Synchronized video (for kinematic analysis) and electromyograms (EMGs; for motor patterns) were collected from each turtle, yielding eight to 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 supplementary material Table S1); these data were supplemented with EMG data for a single muscle (latissimus dorsi) from a fourth individual. We synchronized those EMG data based on the start of humeral elevation and completion of humeral depression (see supplementary material Table S2).

To facilitate digitization of animal movement from videos, nontoxic white dots provided high-contrast points on the following 14 anatomical landmarks (Fig. 1): anterior tip of the nose; shoulder; elbow; digits 1, 3 and 5 on the foreflipper; two landmarks on the

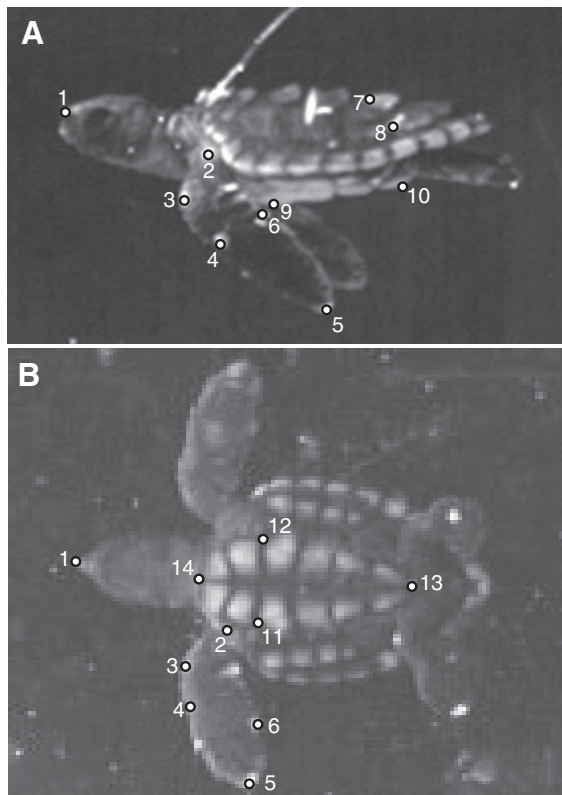


Fig. 1. Representative still images from lateral (A) and ventral (B) videos showing landmarks digitized for kinematic analysis. Landmarks common to both views include: 1, tip of the nose; 2, shoulder; 3, elbow; 4, digit 1; 5, digit 3 (tip of flipper); and 6, digit 5. Additional lateral landmarks (A) include: 7, high landmark on carapace; 8, low landmark on carapace; 9, anterior point on plastron; and 10, posterior point on plastron. Additional ventral landmarks (B) include: 11, point on left side of plastron; 12, point on right side of plastron; 13, posterior point on plastron along midline; and 14, anterior point on plastron along midline.

carapace; an anterior and posterior point on the lateral edge of the plastron; and anterior, posterior, right and left points on the plastron. 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 version 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 us 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.

Standard conventions for limb angle definitions from a previous study (Rivera and Blob, 2010) were applied. Briefly, a humeral protraction/retraction angle of 0 deg indicates that the humerus is perpendicular to the midline of the turtle, whereas an angle of 90 deg indicates a fully protracted forelimb with the distal end of the humerus directed anteriorly (an angle of -90 deg would indicate a fully retracted forelimb with the distal tip of the humerus directed

posteriorly). A humeral elevation/depression angle of 0 deg 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 deg (although not anatomically possible) would indicate a fully flexed elbow (i.e. humerus perfectly parallel to radius and ulna), whereas 180 deg 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 deg 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 deg, and a perfect low-drag orientation of the flipper blade is indicated by an angle of 0 deg.

#### Collection and analysis of electromyographic data

Concurrent with video acquisition, electromyography (EMG) was used to measure muscle-firing patterns of target forelimb muscles. Following previously established protocols (Loeb and Gans, 1986; Westneat and Walker, 1997; Gillis and Blob, 2001; Blob et al., 2008; Rivera and Blob, 2010; Schoenfuss et al., 2010), bipolar fine-wire electrodes (0.05 mm diameter; insulated stainless steel; 0.5 mm 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. Because of the protected status of loggerhead sea turtles, we were 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 (two to three) 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 (60 Hz notch filter, 30 Hz–6 kHz 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 EMG 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 (Rivera and Blob, 2010; Schoenfuss et al., 2010).

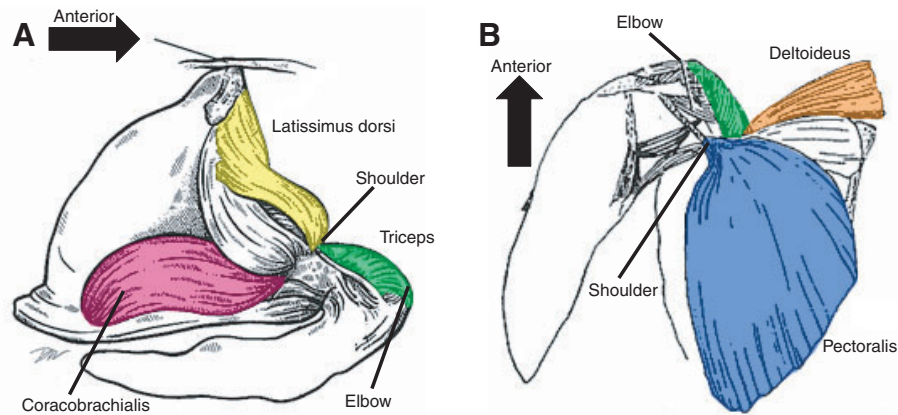


Fig. 2. Illustration showing the five target muscles from which electromyographic data were collected. Positions of the shoulder and elbow are indicated; arrows indicate the anterior of the turtle. (A) Lateral view of the right forelimb musculature of *Caretta caretta* (modified from Wyneken, 2001). (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). The coracobrachialis (pink) is situated posterior to the humerus and expected to retract the forelimb. The most ventral target muscle, the 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. The 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. The 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.

We focused on five target muscles (Fig. 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 originates on the dorsal surface of the coracoid and inserts on the medial process of the humerus. Because of its position posterior to the humerus, the coracobrachialis is expected to retract the forelimb. The pectoralis is a large, triangular sheet that originates from the anterior plastron and extends widely from approximately the plastral midline to converge and insert *via* a tendon 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, originating from the carapace and scapula and inserting on the dorsal surface of the humerus just distal to the head, and is predicted to protract and elevate the limb. The deltoideus originates on the scapula, ventral to the origin of the latissimus dorsi, along the scapula's ventral half, on the acromion process, and from the anterior plastron close to its midline. It is a complexly pinnate muscle that extends across the shoulder joint inserting on the lateral process of the humerus. Its predicted actions are humeral protraction and elevation. Finally, the triceps complex is located on the extensor surface of the arm, running from the shoulder joint to the elbow (originating from the humerus and scapula and inserting on the proximal ulna), 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 were calculated for all swimming trials. Muscle activity variables include, for each muscle: (1) onset, (2) offset and (3) duration. We also evaluated the following eight groups of kinematic variables: (1) maximum protraction, retraction, elevation and depression of the humerus; (2) maximum elbow extension and flexion; (3) anteroposterior and dorsoventral excursion of the humerus; (4) elbow excursion; (5) percentage of the cycle at which maximum elbow extension occurs;

(6) percentage of the limb cycle at which a switch from elevation to depression occurs; (7) percentage of the limb cycle at which a switch from protraction to retraction occurs; and (8) 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 mean of the maximum values calculated for all limb cycles may be masked (appear lower) in average kinematic profiles. We compare our data for loggerheads with those previously published for rowing-style swimming in sliders (Rivera and Blob, 2010) 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. We used Systat (version 12, Chicago, IL, USA) for all statistical analyses, and  $P < 0.05$  as the criterion for significance.

To determine whether swimming forelimb kinematics and motor patterns differ between *C. caretta* and *T. scripta* [presented in Rivera and Blob (Rivera and Blob, 2010)], we conducted two-way mixed-model nested ANOVAs, 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 for each variable, with one set performed on data from each kinematic variable (Table 1) and one on each muscle timing variable (Table 2). In tabular data summaries, we provide d.f. and  $F$ -values to clarify the potential effects of making multiple comparisons. Data are presented as means  $\pm$  s.e.m.

#### RESULTS

Timing of muscle activity relative to limb motion was measured for eight to 14 swimming trials from each of the four sea turtles, with three-dimensional kinematics calculated from three of the four animals (see supplementary material Table S1). 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

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

<sup>a</sup>Values represent the total angular excursion.

\**P*<0.05; \*\**P*<0.01; \*\*\**P*<0.001.

EMG data for *C. caretta* and published data for *T. scripta* (Rivera and Blob, 2010) (see supplementary material Table S2). A summary of sample sizes from each individual, by species, is given for statistical analyses (see supplementary material Tables S1, S2). Limb cycle durations were similar for swimming *C. caretta* (0.55±0.01 s) and *T. scripta* (0.46±0.01 s), facilitating comparisons between the species.

#### 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 *C. 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 *T. scripta*, in which the limb cycle was defined as humeral protraction followed by retraction. Although the limb cycle was defined differently for *C. caretta* and *T. scripta*, 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 *C. caretta* is characterized by humeral elevation and, to a lesser degree, protraction, which both reach a single peak before being followed by extensive humeral depression accompanied by a small degree of retraction (Fig. 3A,B). The elbow of *C. 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 *C. caretta* occurs at 52±2.5% of the limb cycle, which is significantly later than that observed in *T. scripta* at 42±1.0% of the limb cycle (Fig. 3A; Table 1). Although the range of dorsoventral humeral motion is far greater in *C. caretta* (61±4.5 deg versus 28±0.7 deg in *T. scripta*), it is achieved primarily by much greater humeral depression in *C. caretta* (-51±2.6 deg versus -8±0.6 deg in *T. scripta*; Fig. 3A; Table 1). Maximum humeral elevation does not differ significantly between the species, though *T. scripta* primarily holds the humerus above the horizontal plane and *C. caretta* primarily holds the humerus below the horizontal plane (Fig. 3A; Table 1).

Peak humeral protraction differs significantly between *C. caretta* (64±2.2 deg) and *T. scripta* (115±1.4 deg; Fig. 3B; Table 1). In addition, the humerus of *C. caretta* is retracted far less (to an angle

26±2.0 deg anterior to the transverse axis, for an excursion averaging 38±2.4 deg) than that of *T. scripta* (to an angle only 8±0.8 deg anterior to the transverse axis, for an excursion averaging 107±1.7 deg; Fig. 3B; Table 1). Thus, the range of anteroposterior motion of the humerus in *T. scripta* during rowing is much greater than that observed in *C. caretta* during flapping (Fig. 3B; Table 1). In fact, just as the humerus of *T. scripta* moves in a very narrow dorsoventral range of motion, so too is the humerus of *C. caretta* greatly restricted in its range of anteroposterior motion (Fig. 3A,B; Table 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 (*C. caretta*=44±2.9% and *T. scripta*=43±0.6% of the limb cycle; Fig. 3B; Table 1). Peak humeral protraction in *C. 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 *C. caretta* and *T. scripta* (Fig. 3C). Swimming *C. caretta* extend 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. 3C). Swimming *T. scripta* 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. 3C). It appears that the movement patterns are quite similar, though shifted temporally so they are approximately a quarter-cycle out of phase. *Caretta caretta* holds the elbow much straighter (more extended) than *T. scripta* throughout the limb cycle (Fig. 3C). Although 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. 3C; Table 1).

The orientation of the forefoot relative to the direction of travel (or the direction of water flow) differs between *C. caretta* and *T. scripta* (Fig. 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

Table 2. Mean values and standard errors for EMG timing variables (% of the limb cycle) and *F*-values for the main effect of species

Variable	<i>Caretta caretta</i>	<i>Trachemys scripta</i>	<i>F</i>	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 <sup>a</sup>				
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 <sup>a</sup>				
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 <sup>b</sup>				
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 <sup>c</sup>				
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 ( <i>versus</i> Burst 1 in <i>T. scripta</i> ) <sup>d</sup>				
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
Triceps ( <i>versus</i> Burst 2 in <i>T. scripta</i> ) <sup>d</sup>				
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.

<sup>a</sup>*Caretta 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 with both Bursts 1 and 2 in *T. scripta*.

<sup>b</sup>The 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'.

<sup>c</sup>*Caretta 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 values transformed by subtracting 100. For *T. scripta*, onset is the start of 'Burst 1' and offset is the end of 'Burst 2'.

<sup>d</sup>*Caretta 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 with both discrete bursts of activity observed in *T. scripta*. For *T. scripta*, statistical comparison of onset of Burst 1 uses values transformed by adding 100; comparison of offset of Burst 2 uses values transformed by subtracting 100.

Total duration is the combined early and late durations, though not all trials showed both (see supplementary material Table S1).

Brackets indicate transposed values.

\**P*<0.05; \*\**P*<0.01; \*\*\**P*<0.001.

here for forefoot orientation in both species are based on position of the digits and the elbow. Here we deviate slightly from Rivera and Blob (Rivera and Blob, 2010) in the methods used for calculation and plotting of this variable so we can compare this variable between the species using the same metrics. The forefoot of *C. caretta* is held in an increasingly low-drag orientation throughout the first half of the limb cycle; at the start of humeral depression, *C. 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. 3D). In contrast, *T. scripta* feathers the forefoot (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 [similar to previous reports (Pace et al., 2001; Rivera and Blob, 2010)]. *Trachemys scripta* shows a second peak of high-drag forefoot orientation roughly two-thirds of the way through the retraction phase (Fig. 3D), and ends with the palmar surface of the

forefoot directed dorsally. Although the general pattern of forefoot orientation differs between the species in that *C. caretta* exhibits only a single peak, *versus* two for *T. scripta*, 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. 3D). Although 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), *T. scripta* feathers the forefoot less so that it is in a significantly higher drag position than the forefoot of *C. caretta* (Table 1).

There are strong differences between flapping and rowing kinematics for these species of swimming turtles (Table 1). First, flapping in *C. caretta* is characterized by a large range of dorsoventral humeral motion and a restricted amount of anteroposterior movement whereas rowing in *T. scripta* is typified by a large amount of anteroposterior motion and limited dorsoventral

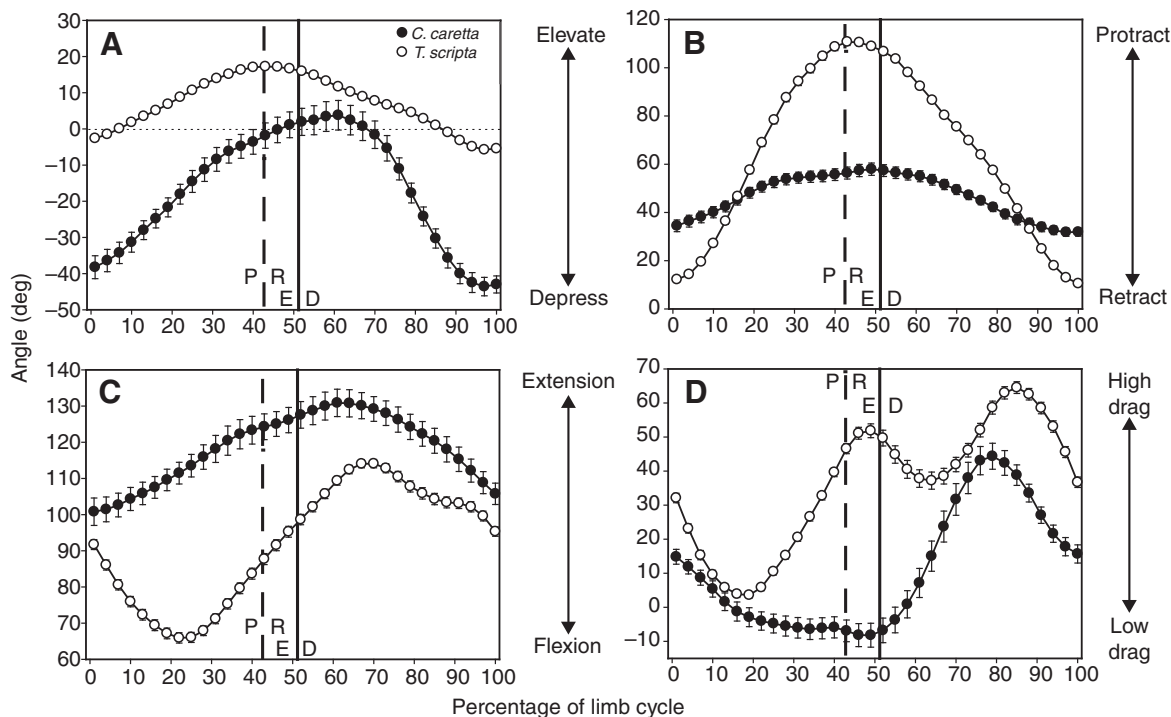


Fig. 3. Mean kinematic profiles for *Caretta caretta* (filled symbols) and *Trachemys scripta* (open symbols) during swimming. Data for *T. scripta* were provided by Rivera and Blob (Rivera and Blob, 2010). Each trial from *C. caretta* was normalized to the same duration, with values interpolated to 100 points, representing the complete limb cycle. The limb cycle for *C. caretta* is defined as elevation of the humerus followed by depression; the limb cycle for *T. scripta* is defined as protraction of the humerus followed by retraction. Mean angle values  $\pm$  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). Angles greater than zero indicate elevation above the horizontal (distal end above proximal end) and angles less than zero 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 deg indicates that the humerus is perpendicular to the midline of the turtle, whereas an angle of 90 deg indicates a fully protracted forelimb with the distal end of the humerus directed anteriorly (an angle of  $-90$  deg would indicate a fully retracted forelimb with the distal tip of the humerus directed posteriorly). (C) Elbow flexion and extension. An angle of 0 deg indicates complete flexion, whereas 180 deg 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 deg from each value. Data originally reported for *T. scripta* forefoot orientation in Rivera and Blob (Rivera and Blob, 2010) 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 deg, and a perfect low-drag orientation of the forefoot paddle is indicated by a feathering angle of 0 deg.

movement. Second, the greater dorsoventral range of motion during flapping is accomplished through an increase in humeral depression, but without a change in humeral elevation. The greater range of anteroposterior motion observed in rowing is achieved through both greater humeral protraction and retraction. Third, the timing of maximum humeral elevation differs between the species, but within each species is roughly coincident with the timing of maximum protraction. Fourth, although the amount of elbow motion (excursion angle) is similar between the species, their elbows move through different arcs, with *C. caretta* consistently holding the elbow in a more extended position. Finally, rowing in *T. scripta* is characterized by a much higher-drag orientation of the forefoot during the second phase of the limb cycle than is seen in *C. caretta*.

#### Patterns of muscle activation: flapping versus rowing

Four of our five pectoral girdle target muscles in *C. caretta* were active during portions of the limb cycle as predicted based on their anatomical positions. Among predicted humeral retractors and depressors, the coracobrachialis exhibits a single burst of activity during most of humeral depression and retraction in both *C. caretta*

and *T. scripta*, the timing of which does not differ between the species (Fig. 4; Table 2). In contrast, the other predicted humeral retractor, the pectoralis, exhibits one burst of activity in *C. caretta*, but two bursts of activity in *T. scripta* (Fig. 4). The early burst of pectoralis activity in *T. scripta* is variable (Rivera and Blob, 2010) and, when present, always occurs during protraction/elevation. In contrast, the later burst of pectoralis activity in *T. scripta* always occurred during retraction/depression, similar to the single burst observed for *C. caretta* (Fig. 4). The single burst in *C. caretta* was compared with each of the two bursts of activity seen in *T. scripta* (Table 2). It differed significantly in both onset and offset of activity when compared with the *T. scripta* (variable) early burst; when compared with the later burst, it only differed in offset of activity (Table 2).

Among humeral protractors and elevators, one muscle (latissimus dorsi) exhibits a similar pattern between the species, but another (deltoideus) differs substantially (Fig. 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. 4). Because our definition of the



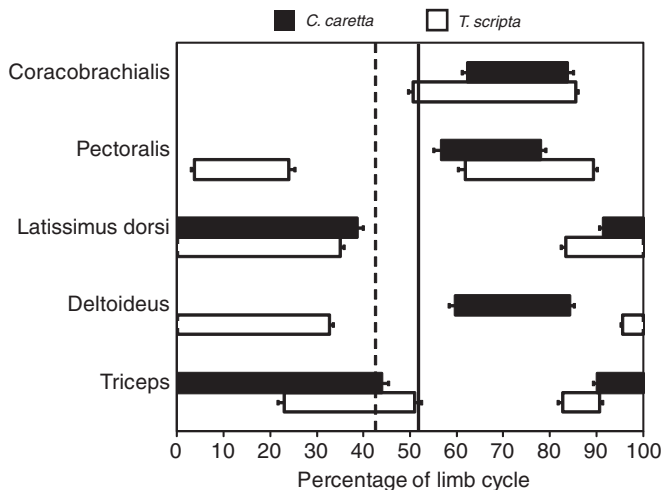


Fig. 4. Bar plot showing patterns of forelimb muscle activation during swimming for *C. caretta* (flapping, filled bars) and *T. scripta* (rowing, open bars). Data for *T. scripta* were provided by Rivera and Blob (Rivera and Blob, 2010). Bars illustrate mean ranges ( $\pm$  s.e.m. for onset and offset) for the period of activity for each muscle. Filled 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%.

limb cycle divides these continuous bursts into two portions for graphic presentation, we term these portions as ‘early’ and ‘late bursts’ or ‘Burst 1’ and ‘Burst 2’, respectively (Rivera and Blob, 2010), using quotation marks 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 *C. caretta* and *T. scripta* (Table 2).

The deltoideus, the other focal humeral protractor and elevator, displays a very different pattern between the two study species (Fig. 4). *Caretta caretta* shows just one discrete burst of deltoideus activity, occurring during depression and retraction (Fig. 4). In contrast, *T. scripta* 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. 4). Onset of deltoideus activity in *T. scripta* is the start of ‘Burst 2’ and offset is the end of ‘Burst 1’ (Rivera and Blob, 2010). To facilitate comparison of offset of deltoideus activity, offset in *C. caretta* was transformed by subtracting 100. Comparisons of timing variables indicate significant differences in burst onset and offset, but not the duration of activity (Table 2).

The triceps complex also shows different patterns of activation between the two species. *Caretta 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. 4); this corresponds with elbow extension (Fig. 3C). *Trachemys scripta*, 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. 4). Although the early triceps burst was always present in *T. scripta*, the later burst was variable (Rivera and Blob, 2010).

To facilitate statistical comparisons between the species, onset of triceps activity in *C. caretta* is the start of ‘Burst 2’ and offset is the end of ‘Burst 1’; onset of the first burst in *T. scripta* was transformed by adding 100, whereas offset of the variable second burst was transformed by subtracting 100 (Table 2). Triceps activity in *C. caretta* starts significantly earlier and has a significantly longer duration than the first burst of activity seen in *T. scripta*, though offset did not differ (Fig. 4; Table 2). Offset of triceps activity in *C. caretta* occurred significantly later and lasted longer than the variable second burst of *T. scripta*, but onset did not differ (Fig. 4; Table 2).

## DISCUSSION

### Kinematic comparison of flapping and rowing

Our study supports the interpretation that the evolution of the derived trait of flapping propulsion involved changes in a wide range of kinematic features beyond the primary plane of humeral motion (Licht et al., 2010). The primary differences 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; Rivera and Blob, 2010) are supported by the details and the trends in our data (Fig. 3A,B). Dorsoventral humeral motion is much greater in flapping, whereas anteroposterior motion is much greater during rowing. Humeral motions outside of these predominant axes 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 *C. caretta* is achieved through a much greater degree of humeral depression than is usual for *T. scripta*, whereas the larger anteroposterior range of motion in *T. scripta* is accomplished through both increased humeral protraction and retraction relative to that accomplished by *C. caretta*. Additionally, *T. scripta* generally moves the forelimb through most of its cycle held slightly above the horizontal whereas *C. caretta* generally positions the forelimb below the horizontal axis, with the majority of dorsoventral movement occurring below this plane (Fig. 3A). Although 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 to average limb orientation, the differences in muscle activation between the species suggest that structural differences are not the sole factor driving species-specific humeral movements during rowing or flapping across turtles.

Elbow kinematics also differ between the species (Fig. 3C). Rowing, in *T. scripta*, is accomplished with a limb that is first flexed and then extended at the elbow, before being flexed again. Flapping, in *C. 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. 3C). Although the angular excursion of the elbow does not differ between species, maximum extension and flexion do differ because the forelimb of *C. caretta* is more fully extended (i.e. held straighter) throughout the limb cycle (Fig. 3C; Table 1). Although humeral 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 we observed.

Forefoot or flipper blade orientation shows both similarities and differences between the species. Although total forefoot feathering excursion does not differ, a higher-drag orientation of the forefoot is observed in rowing *T. scripta* (Fig. 3D; Table 1), consistent with

characterizations of these species as 'drag-based' versus 'lift-based' swimmers (e.g. Vogel, 1994; Wyneken, 1997). Yet, both species 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) whereas the pattern observed in *T. scripta* may simply mark the switch between thrust (retraction) and recovery (protraction) strokes.

#### Patterns of muscle activation during flapping-style swimming in *C. caretta*

Anatomical positions were good predictors of the muscle function observed in four of our five pectoral girdle target muscles in *C. caretta*. 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. 4). However, the deltoideus exhibited one discrete burst of activity during humeral depression and retraction; this is the opposite of when it was predicted to be active based on its anatomical position (Walker, 1973). The deltoideus was predicted to act primarily as a humeral protractor and elevator in sea turtles (Walker, 1973), yet we found that its primary role is more likely to be as a modifier constraining humeral retraction during depression. *Caretta 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, although the configuration of the pectoral girdle musculature is quite similar in all turtles with regard to origin, insertion and relative size (Walker, 1973), sea turtles (including *C. caretta*) possess an enlarged pectoralis relative to that of freshwater turtles (Walker, 1973; Wyneken, 2001). The larger pectoralis of *C. caretta* likely contributes to its substantial humeral depression, but also retracts the humerus. The simultaneous protraction generated by the deltoideus as it fires during the forelimb downstroke (Fig. 4) should constrain the degree of humeral retraction produced by the pectoralis, thereby resulting in depression of the humerus with very little anteroposterior movement (Fig. 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 our comparison of swimming motor patterns in flapping *C. caretta* and rowing *T. scripta* provides a composite of support for the neuromotor conservation hypothesis and also evidence suggesting that 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 *C. caretta* and *T. scripta* swim, these two muscles display conserved activity patterns, lending support to the hypothesis of neuromotor conservation.

In contrast, the pattern of activation for other muscles shows marked differences that suggest a lack of conservation. Our comparison reveals differences not only in the timing of muscle bursts (deltoideus), but also in the number of bursts (pectoralis and

triceps complex) (Fig. 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. Although the deltoideus serves as a strong humeral protractor during rowing-style swimming in *T. scripta*, in *C. caretta* it stabilizes and minimizes anteroposterior movements of the humerus through simultaneous activation with the pectoralis. This activity could help to counter potential retraction generated by the pectoralis during the downstroke of sea turtles, resulting in depression of the forelimb with limited anteroposterior movement during flapping. In addition, because the flippers of sea turtles are much larger than the forefeet of sliders, it is possible that the reaction force of oncoming water acting on swimming sea turtles might impose greater drag on their forelimbs than on those of sliders, and that such additional drag might also be countered by deltoideus activity. Thus, through a simple shift in activation timing, the functional role of the 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. Although the *T. scripta* pectoralis 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), in *C. caretta* it exhibits a single burst of activity during depression/retraction (consistent with predictions based on its anatomical position; Fig. 4). Comparisons of the two bursts in *T. scripta* with the single burst in *C. caretta* show significant differences in timing relative to the first burst (Table 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 *C. caretta* appears to be conserved and homologous to the second burst of activity in *T. scripta*, but not the first. Why does *C. caretta* not display the same variable pectoralis burst thought to act in shoulder stabilization in *T. scripta*? 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 *T. scripta* occurs (during slight elevation and protraction) is coincident with the *C. caretta* upstroke (elevation and slight protraction). The enlarged pectoralis of *C. 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, the *T. scripta*-like Burst 1 activity would likely be selected against. Although the primary pattern of activity for the 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. It is also possible that simultaneous activation of the deltoideus and the pectoralis early in the limb cycle (i.e. during elevation/protraction) of *T. scripta* acts to stabilize the limb against the increasing drag incurred by the forward-moving forefoot at this time (Figs 3, 4).

Finally, the triceps complex differs in both timing and number of muscle bursts between flapping and rowing species. Although *T. scripta* shows two bursts of triceps activity, *C. caretta* shows one long continuous burst. The early triceps burst in *T. scripta* occurs during elbow extension and was always present, whereas the later burst was variable and may act in elbow stabilization (Rivera and Blob, 2010). Although timing of onset was similar, triceps activity in *C. caretta* differs significantly in offset and duration from the variable second burst in *T. scripta* (Table 2). When compared with

the early burst in *T. scripta* (the burst playing a similar role in elbow extension), we found significant differences in onset and duration (Table 2). In spite of similar primary function in both species (elbow extension), the substantial kinematic differences in the pattern of elbow extension between flapping and rowing (Fig. 3C) appear to be controlled by a difference in the pattern of activation of the triceps.

We conclude that the evolution of flapping-style swimming in sea turtles, as exemplified by *C. caretta*, is a case of a new locomotor behavior accomplished through changes in both structure of the forelimb as well as some changes in the patterns of activation of forelimb muscles. We found the activity of several muscles (coracobrachialis, latissimus dorsi, and pectoralis with respect to its late burst) to be conserved between the species, but one muscle, the deltoideus, has taken on a new role in flapping *C. caretta*. Additionally, 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. 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, although our study provides partial support for the hypothesis of neuromotor conservation, it also identifies notable exceptions.

Examination of additional species will likely determine whether motor activation patterns are similarly modified across a broader range of locomotor behavior. Although 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 that are even more restricted to a horizontal plane (Pace et al., 2001). Additionally, *Carettochelys insculpta*, the Australian pig-nosed 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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#### REFERENCES

- Barley, A. J., Spinks, P. Q., Thomson, R. C. and Shaffer, H. B. (2010). Fourteen nuclear genes provide phylogenetic resolution for difficult nodes in the turtle tree of life. *Mol. Phylogenet. Evol.* **55**, 1189-1194.
- Blake, R. W., Chatters, L. M. and Domenici, P. (1995). Turning radius of yellowfin tuna (*Thunnus albacares*) in unsteady swimming manoeuvres. *J. Fish Biol.* **46**, 536-538.
- Blob, R. W., Rivera, A. R. V. and Westneat, M. W. (2008). Hindlimb function in turtle locomotion: limb movements and muscular activation across taxa, environment, and ontogeny. In *Biology of Turtles* (ed. J. Wyneken, M. H. Godfrey and V. Bels), pp. 139-162. Boca Raton: CRC Press.
- Davenport, J., Munks, S. A. and Oxford, P. J. (1984). A comparison of the swimming of marine and freshwater turtles. *Proc. R. Soc. Lond. B* **220**, 447-475.
- Dial, K. P., Goslow, G. E. and Jenkins, F. A. (1991). The functional anatomy of the shoulder in the European starling (*Sturnus vulgaris*). *J. Morphol.* **207**, 327-344.
- Dougherty, E., Rivera, G., Blob, R. and Wyneken, J. (2010). Hydrodynamic stability in posthatching loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles. *Zoology* **113**, 158-167.
- Ernst, C. H. and Lovich, J. E. (2009). *Turtles of the United States and Canada*, 2nd edn. Baltimore: The Johns Hopkins University Press.
- Fish, F. E. (1996). Transitions from drag-based to lift-based propulsion in mammalian swimming. *Am. Zool.* **36**, 628-641.
- Fish, F. E. (2002). Balancing requirements for stability and maneuverability in cetaceans. *Integr. Comp. Biol.* **42**, 85-93.
- Fish, F. E. and Nicasastro, A. J. (2003). Aquatic turning performance by the whirligig beetle: constraints on maneuverability by a rigid biological system. *J. Exp. Biol.* **206**, 1649-1656.
- Garland, T. and Adolph, S. C. (1994). Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiol. Zool.* **67**, 797-828.
- Gillis, G. B. and Blob, R. W. (2001). How muscles accommodate movement in different physical environments: aquatic vs. terrestrial locomotion in vertebrates. *Comp. Biochem. Physiol.* **131A**, 61-75.
- Goslow, G. E., Dial, K. P. and Jenkins, F. A. (1989). The avian shoulder: an experimental approach. *Am. Zool.* **29**, 287-301.
- Goslow, G. E., Wilson, D. and Poore, S. O. (2000). Neuromuscular correlates to the evolution of flapping flight in birds. *Brain Behav. Evol.* **55**, 85-99.
- Gosnell, J. S., Rivera, G. and Blob, R. W. (2009). A phylogenetic analysis of sexual size dimorphism in turtles. *Herpetologica* **65**, 70-81.
- Hedrick, T. L. (2008). Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspir. Biomim.* **3**, 034001.
- Jenkins, F. A. and Goslow, G. E. (1983). The functional anatomy of the shoulder of the Savannah monitor lizard (*Varanus exanthematicus*). *J. Morphol.* **175**, 195-216.
- Jenkins, F. A. and Wejls, W. A. (1979). The functional anatomy of the shoulder of the Virginia opossum *Didelphis virginiana*. *J. Zool.* **188**, 379-410.
- Lauder, G. V. and Reilly, S. M. (1996). The mechanistic bases of behavioral evolution: a multivariate analysis of musculoskeletal function. In *Phylogenies and the Comparative Method in Animal Behavior* (ed. E. P. Martins), pp. 104-137. New York: Oxford University Press.
- Licht, S. C., Wibawa, M. S., Hover, F. S. and Triantafyllou, M. S. (2010). In-line motion causes high thrust and efficiency in flapping foils that use power downstroke. *J. Exp. Biol.* **213**, 63-71.
- Loeb, G. E. and Gans, C. (1986). *Electromyography for Experimentalists*. Chicago: The University of Chicago Press.
- Pace, C. M., Blob, R. W. and Westneat, M. W. (2001). Comparative kinematics of the forelimb during swimming in red-eared slider (*Trachemys scripta*) and spiny softshell (*Apalone spinifer*) turtles. *J. Exp. Biol.* **204**, 3261-3271.
- Peters, S. E. and Goslow, G. E. (1983). From salamanders to mammals: continuity in musculoskeletal function during locomotion. *Brain Behav. Evol.* **22**, 191-197.
- Reilly, S. M. and Lauder, G. V. (1992). Morphology, behavior, and evolution: comparative kinematics of aquatic feeding in salamanders. *Brain Behav. Evol.* **40**, 182-196.
- Renous, S. and Bels, V. (1993). Comparison between aquatic and terrestrial locomotion of the leatherback sea turtle (*Dermochelys coriacea*). *J. Zool.* **230**, 357-378.
- Renous, S., Lapparent de Broin, F., Depecker, M., Davenport, J. and Bels, V. (2008). Evolution of locomotion in aquatic turtles. In *Biology of Turtles* (ed. J. Wyneken, M. H. Godfrey and V. Bels), pp. 97-138. Boca Raton: CRC Press.
- Rivera, A. R. V. and Blob, R. W. (2010). 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. *J. Exp. Biol.* **213**, 3515-3526.
- Rivera, G., Rivera, A. R. V., Dougherty, E. E. and Blob, R. W. (2006). Aquatic turning performance of painted turtles (*Chrysemys picta*) and functional consequences of a rigid body design. *J. Exp. Biol.* **209**, 4203-4213.
- Schoenfuss, H. L., Roos, J. D., Rivera, A. R. V. and Blob, R. W. (2010). Motor patterns of distal hind limb muscles in walking turtles: implications for models of limb bone loading. *J. Morphol.* **271**, 1527-1536.
- Smith, K. K. (1994). Are neuromotor systems conserved in evolution? *Brain Behav. Evol.* **43**, 293-305.
- Stokes, L., Wyneken, J., Crowder, L. and Marsh, J. (2006). The influence of temporal and spatial origin on size and early growth rates in captive loggerhead sea turtles (*Caretta caretta*) in the United States. *Herpetol. Conserv. Biol.* **1**, 71-80.
- Vogel, S. (1994). *Life in Moving Fluids*. Princeton, NJ: Princeton University Press.
- Walker, J. A. (1998). Estimating velocities and accelerations of animal locomotion: a simulation experiment comparing numerically different algorithms. *J. Exp. Biol.* **201**, 981-995.
- Walker, J. A. (2000). Does a rigid body limit maneuverability? *J. Exp. Biol.* **203**, 3391-3396.
- Walker, J. A. and Westneat, M. W. (2000). Mechanical performance of aquatic rowing and flying. *Proc. R. Soc. Lond. B* **267**, 1875-1881.
- Walker, W. F., Jr (1971). Swimming in sea turtles of the family Cheloniidae. *Copeia* **1971**, 229-233.
- Walker, W. F., Jr (1973). The locomotor apparatus of Testudines. In *Biology of the Reptilia, Vol. 4, Morphology D* (ed. C. Gans and T. S. Parsons), pp. 1-100. London: Academic Press.
- Westneat, M. W. and Wainwright, P. C. (1989). Feeding mechanism of *Epibulus insidiator* (Labridae, Teleostei): evolution of a novel functional system. *J. Morphol.* **202**, 129-150.
- Westneat, M. W. and Walker, J. A. (1997). Motor patterns of labriform locomotion: kinematic and electromyographic analysis of pectoral fin swimming in the labrid fish *Gomphosus varius*. *J. Exp. Biol.* **200**, 1881-1893.
- Wyneken, J. (1997). Sea turtle locomotion: mechanisms, behavior, and energetics. In *The Biology of Sea Turtles* (ed. P. L. Lutz and J. A. Musick), pp. 165-198. Boca Raton: CRC Press.
- Wyneken, J. (2001). *Guide to the Anatomy of Sea Turtles*, 172 pp. NOAA Tech Memo NMFS-SEFSC-470. Miami: US Department of Commerce.
- Zug, G. R. (1971). Buoyancy, locomotion, morphology of the pelvic girdle and hind limb, and systematics of cryptodiran turtles. *Misc. Publ. Mus. Zool. Univ. Mich.* **142**, 1-98.