Forelimb muscle function in pig-nosed turtles, *Carettochelys insculpta*: testing neuromotor conservation between rowing and flapping in swimming turtles

Angela R. V. Rivera and Richard W. Blob

Department of Biological Sciences, Clemson University, Clemson, SC 29634, USA

Changes in muscle activation patterns can lead to new locomotor modes; however, neuromotor conservation—the evolution of new forms of locomotion through changes in structure without concurrent changes to underlying motor patterns—has been documented across diverse styles of locomotion. Animals that swim using appendages do so via rowing (anteroposterior oscillations) or flapping (dorsoventral oscillations). Yet few studies have compared motor patterns between these swimming modes. 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 hybrid style with superficial similarity to flapping by sea turtles and characterized by increased dorsoventral motions of synchronously oscillated forelimbs that have been modified into flippers (*Carettochelys insculpta*). We compared forelimb motor patterns in four species of turtle (two rowers, *Apalone ferox* and *Trachemys scripta*; one flapper, *Caretta caretta*; and *Carettochelys*) and found that, despite kinematic differences, motor patterns were generally similar among species with a few notable exceptions: specifically, presence of variable bursts for pectoralis and triceps in *Trachemys* (though timing of the non-variable pectoralis burst was similar), and the timing of deltoideus activity in *Carettochelys* and *Caretta* compared with other taxa. The similarities in motor patterns we find for several muscles provide partial support for neuromotor conservation among turtles using diverse locomotor styles, but the differences implicate deltoideus as a prime contributor to flapping limb motions.

1. 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. Although 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 [1]. Rowing and flapping have been documented among diverse taxa, including fishes [1–3], turtles [4–6], birds [7] and mammals [8,9]. 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 behaviours 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’ [10]. While evidence for neuromotor conservation is found across terrestrial and aerial locomotor modes [11–13], 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 [14]. Species of aquatic turtles swim via rowing or flapping; all but one freshwater species (there are over 200) swim using asynchronous rowing of paddle-shaped forelimbs and hindlimbs, whereas all marine turtles (seven species) swim using synchronous flapping of forelimbs that are 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, kinematically, 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 resembles rowing in many respects [15]. However, angles-of-attack indicate that Carettochelys are hydrodynamically more concordant with flappers during protraction/elevation [15]. A recent examination of forelimb motor patterns in rowing Trachemys scripta (hereafter ‘Trachemys’, family Emydidae) and flapping Caretta caretta (hereafter ‘Caretta’, family Chelonidae) 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 [6].

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 flippers, with whose locomotor style Carettochelys shares some similarities. To do this, we measured forelimb motor patterns in swimming Carettochelys, as well as Apalone ferox (hereafter ‘Apalone’, family Trionychidae), a specialized ruler and member of the sister taxon to the monotypic Carettochelyidae [16], thus providing a phylogenetic comparison. We compare these results with previous measurements from a generalized rower (Trachemys) and a flapping sea turtle (Caretta). By comparing motor patterns from four of the 13 families containing aquatic species, we were 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 [15]), flapping and a unique hybrid style of locomotion that displays similarities with both freshwater rowers and marine flappers.

2. Material and methods

(a) Animals
Access to two pig-nosed turtles, C. insculpta (carapace length = 23.8 ± 1.8 cm), and nine Florida softshell turtles, A. ferox (carapace length = 15.1 ± 1.1 cm), was provided by a commercial vendor. The number of Carettochelys was limited owing to infrequent availability of this rare species. Turtles were housed in stock tanks [15].

(b) Collection and analysis of electromyography data
Bipolar stainless steel fine-wire electrodes (0.05 mm diameter, California Fine Wire Co., USA) were implanted percutaneously into target muscles of the left forelimb of Carettochelys (see [6] for details) and Apalone (see [5] for details) to generate data for comparison to other species. Protocols differed only slightly for the species; in particular, Carettochelys received local anaesthetic (lidocaine) at implant sites and were tested the same day, whereas Apalone was anaesthetized with ketamine prior to implants and tested the following day. Electromyography (EMG) data (5000 Hz) were synchronized with kinematics (collected using biplanar high-speed (100 Hz) video with high-contrast joint markers; detailed in [15]) and analysed in LabVIEW, where muscle timing was determined visually.

We focused on five target muscles, covering all major planes of forelimb motion 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) [17,18].

(c) Statistical analyses
To assess general patterns of muscle function for each species, means and standard errors for each variable were calculated across all swimming trials (see table 1 and electronic supplementary material, table A1). Muscle activity variables include, for each muscle: (i) onset, (ii) offset and (iii) duration. Data for Carettochelys and Apalone were compared with those previously published for Trachemys [5] and Caretta [6] to assess how motor patterns during swimming compare among the species. Inter-specific differences ($p < 0.05$) were evaluated using separate two-factor nested ANOVAs (SYSTAT), with individual nested within species, followed by Tukey’s post hoc tests to evaluate each pairwise species comparison.

3. Results
Forelimb motor patterns for Carettochelys and Apalone were calculated and compared with those published for Caretta and Trachemys (figure 1 and table 1); turtles of each species swim using similar forelimb cycle frequencies [15]. Patterns are very similar across species for humeral retractors. There are statistically significant, but minor, differences in onset of coracobrachialis (Apalone earlier than Caretta) and offset of pectoralis (Caretta earlier than Trachemys and Carettochelys). Trachemys also exhibits a variable (i.e. not always present) 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 marked differences among the species. Flapping Caretta exhibit a single discrete burst of deltoideus activity during humeral depression and retraction (opposite to the predicted action [6,17]), differing significantly in onset and offset from the three freshwater species. Furthermore, deltoideus activity in Carettochelys starts and ends significantly earlier than in Apalone and Trachemys, resulting in significantly decreased duration of activity during protraction in the former. Finally, triceps differs primarily among the species in that Trachemys exhibits two discrete 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.

4. Discussion
Muscles were active during the predicted portions of the limb cycle and showed similar patterns among species with few
Table 1. Mean values and standard errors for EMG timing variables, F-values for the main effect of species and Tukey pairwise mean comparison results. Consistent with our previous work [5,6,15], ‘Burst no. 1’ and ‘Burst no. 2’ indicate early and late phase muscle activity of continuous muscles, rather than discrete bursts. Values are means ± s.e.m. Significant differences in pairwise comparisons are indicated. AF, Apalone ferox; CC, Caretta caretta; CI, Carettochelys insculpta; TS, Trachemys scripta.

<table>
<thead>
<tr>
<th>variable</th>
<th>Trachemys scripta</th>
<th>Apalone ferox</th>
<th>Carettochelys insculpta</th>
<th>Caretta caretta</th>
<th>F-value</th>
<th>d.f.</th>
<th>Tukey results</th>
</tr>
</thead>
<tbody>
<tr>
<td>coracobrachialis onset</td>
<td>51 ± 1.0</td>
<td>42 ± 0.8</td>
<td>no data</td>
<td>62 ± 1.3</td>
<td>5.16*</td>
<td>2.9</td>
<td>AF – CC</td>
</tr>
<tr>
<td></td>
<td>offset</td>
<td>85 ± 0.5</td>
<td>83 ± 0.4</td>
<td>84 ± 1.3</td>
<td>0.83</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>duration</td>
<td>34.9 ± 1.2</td>
<td>42 ± 1.1</td>
<td>21 ± 1.4</td>
<td>3.01</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>pectoralis onset</td>
<td>62 ± 1.5</td>
<td>51 ± 0.6</td>
<td>57 ± 0.9</td>
<td>57 ± 1.7</td>
<td>1.19</td>
<td>3.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>offset</td>
<td>89 ± 0.7</td>
<td>84 ± 0.4</td>
<td>78 ± 1.1</td>
<td>5.30*</td>
<td>3.11</td>
<td>CC – CI; CC – TS</td>
</tr>
<tr>
<td></td>
<td>duration</td>
<td>28 ± 1.3</td>
<td>33 ± 0.7</td>
<td>21 ± 1.3</td>
<td>1.24</td>
<td>3.11</td>
<td></td>
</tr>
<tr>
<td>latissimus dorsi onset</td>
<td>83 ± 1.0</td>
<td>88 ± 0.8</td>
<td>94 ± 1.1</td>
<td>91 ± 0.9</td>
<td>1.60</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>offset</td>
<td>35 ± 0.9</td>
<td>27 ± 1.5</td>
<td>37 ± 1.4</td>
<td>4.36*</td>
<td>3.7</td>
<td>AF – CC</td>
</tr>
<tr>
<td>‘Burst 1’ duration</td>
<td>35 ± 0.9</td>
<td>27 ± 1.5</td>
<td>37 ± 1.4</td>
<td>37 ± 1.2</td>
<td>3.70</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>‘Burst 2’ duration</td>
<td>16 ± 1.0</td>
<td>12 ± 0.8</td>
<td>6 ± 1.1</td>
<td>8 ± 0.9</td>
<td>1.67</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>total duration</td>
<td>51 ± 1.3</td>
<td>36 ± 2.5</td>
<td>41 ± 2.0</td>
<td>44 ± 1.6</td>
<td>1.89</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>deltoideus onset</td>
<td>96 ± 0.4</td>
<td>94 ± 0.3</td>
<td>81 ± 2.2</td>
<td>60 ± 1.3</td>
<td>217.42***</td>
<td>3.10</td>
<td>all but AF – TS</td>
</tr>
<tr>
<td></td>
<td>offset</td>
<td>33 ± 0.9</td>
<td>30 ± 0.8</td>
<td>7 ± 1.2</td>
<td>39.59***</td>
<td>3.12</td>
<td>all but AF – TS</td>
</tr>
<tr>
<td>‘Burst 1’ duration</td>
<td>30 ± 1.1</td>
<td>30 ± 0.8</td>
<td>7 ± 1.2</td>
<td>6.75*</td>
<td>2.10</td>
<td>AF – CI; CI – TS</td>
<td></td>
</tr>
<tr>
<td>‘Burst 2’ duration</td>
<td>4 ± 0.4</td>
<td>6 ± 0.3</td>
<td>18 ± 2.2</td>
<td>26.94***</td>
<td>2.8</td>
<td>AF – CI; CI – TS</td>
<td></td>
</tr>
<tr>
<td>total duration</td>
<td>32 ± 1.7</td>
<td>35 ± 0.8</td>
<td>20 ± 2.3</td>
<td>24 ± 1.4</td>
<td>2.27</td>
<td>3.12</td>
<td></td>
</tr>
<tr>
<td>triceps onset</td>
<td>23 ± 1.3</td>
<td>94 ± 0.4</td>
<td>89 ± 1.5</td>
<td>90 ± 0.8</td>
<td>11.09**</td>
<td>3.11</td>
<td>AF – TS; CC – TS; CI – TS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>offset</td>
<td>51 ± 1.5</td>
<td>39 ± 0.6</td>
<td>38 ± 1.4</td>
<td>44 ± 1.5</td>
<td>1.61</td>
<td>3.12</td>
</tr>
<tr>
<td>‘Burst 1’ duration</td>
<td>---</td>
<td>37 ± 0.7</td>
<td>38 ± 1.4</td>
<td>40 ± 1.4</td>
<td>0.53</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>‘Burst 2’ duration</td>
<td>---</td>
<td>6 ± 0.4</td>
<td>11 ± 1.5</td>
<td>10 ± 0.8</td>
<td>1.93</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>total duration</td>
<td>28 ± 0.9</td>
<td>41 ± 0.8</td>
<td>47 ± 2.5</td>
<td>45 ± 1.8</td>
<td>5.13**</td>
<td>3.12</td>
<td>CC – TS; CI – TS</td>
</tr>
</tbody>
</table>

4 Apalone, Carettochelys and Caretta exhibit one discrete burst that was compared with the presumptive homologous non-variable later burst in Trachemys.
5 Latissimus dorsi shows a continuous burst that spans the retraction/depression to protraction/elevation phase shift.
6 Apalone, Carettochelys and Trachemys each show one continuous burst (onset = start ‘Burst 2’, offset = end ‘Burst 1’) that was compared with the single discrete burst in Caretta (offset = [transposed value, by subtracting 100]).
7 Apalone, Carettochelys and Caretta exhibit one continuous burst (onset = start ‘Burst 2’, offset = end ‘Burst 1’) that was compared with the discrete non-variable early burst observed in Trachemys (onset = [transposed value, by adding 100]).

*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.

exceptions, including two instances of variable bursts in *Trachemys* (pectoralis and triceps) and, most notably, activity of the deltoideus in *Caretta* (figure 1).

Of particular interest, our new results reveal that some differences in motor patterns (i.e. presence or absence of variable bursts) observed between *Trachemys* and *Caretta* [6] are not attributable to their difference in swimming mode (i.e. rowing versus flapping) as previously proposed, because rowing *Apalone* also lack variable pectoralis and triceps bursts and differ 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 (i.e. retraction/depression) [15]. 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 [5].

The primary difference in motor patterns among species occurs in the deltoideus. Rivera et al. [6] conclude that the...
Walker JA. 2002 Functional morphology and virtual

Walker JA, Westneat MW. 2000 Mechanical

References

Figure 1. Bar plot showing mean (± s.e.m.) pattern of forelimb muscle activation during swimming in Carettochelys insculpta (black), Apalone ferox (white), Trachemys scripta (grey, hatched = variable) and Caretta caretta (diagonals). Vertical lines demarcate switch from protraction/elevation to retraction/depression (solid, Apalone/Trachemys; dashed, Carettochelys/Caretta) based on maxima/minima of kinematics; a limb cycle is defined by the major plane of forelimb motion (start of protraction to end of retraction for Apalone, Trachemys and Carettochelys, and elevation followed by depression for Caretta; detailed in [5,6,15]).

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. 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 unique hybrid-style synchronous rowing by Carettochelys is intermediate between rowing and flapping, showing a shift towards the pattern observed in Caretta and concordant with their exhibited bimodal pattern of elevation and depression (see fig. 4b in [15]).

In conclusion, our data show a general trend of conservation of motor patterns among swimming turtles using a variety of locomotor styles, ranging from generalized and specialized rowing to flapping, and including the unique hybrid-style synchronous rowing of Carettochelys. Some variable muscle activity patterns found in more terrestrial Trachemys were absent from highly aquatic species. 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 across aquatic locomotor behaviours but with variation focused in specific muscles. These data suggest that motor patterns may be conserved whenever morphological modifications alone are not sufficient to produce new patterns of motion. Further, these results suggest that evolutionary changes in muscle activation may occur more readily for some muscles (e.g. deltoideus) while illustrating the potential for completely new forms of locomotion to evolve through simple shifts in activation timing of a small subset of muscles.

Acknowledgements. Jill Galan, Danielle Hulsey and Gabriel Rivera assisted with data collection. Experiments were conducted under Clemson University IACUC guidelines (protocols 50110, 2008–013 and 2008–080).

Funding statement. Support provided by Sigma Xi (Grant-in-Aid-of-Research to A.R.V.R.), NSF (IOS-0517340 to R.W.B.) and NIH (2-R01-DC005063-06A1 to E. Peterson, subaward UT10853 to R.W.B.).

References

6. Rivera ARV, Wyneken J, Blob RW. 2011 Forelimb kinematics and motor patterns of swimming loggerhead sea turtles (Caretta caretta): are motor


