

1 **Inferring cost of transport from whole-body kinematics in three sympatric turtle**  
2 **species with different locomotor habits**

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12 **Keywords**

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14 Kinematics; Energy Recovery; Walking; Biomechanics

15

16 **Abstract**

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18 Chelonians are mechanically unusual vertebrates as an exoskeleton limits their body  
19 wall mobility. They generally move slowly on land and have aquatic or semi-aquatic  
20 lifestyles. Somewhat surprisingly, the limited experimental work that has been done  
21 suggests that their energetic costs of transport (CoT) are relatively low. This study  
22 examines the mechanical evidence for CoT in three turtle species that have differing  
23 degrees of terrestrial activity. Our results show that *Apolone* travels faster than the  
24 other two species, and that *Chelydra* has higher levels of yaw. All the species show  
25 poor mean levels of energy recovery, and, whilst there is considerable variation, never  
26 show the high levels of energy recovery seen in cursorial quadrupeds. The mean  
27 mechanical CoT is 2 to 4 times higher than is generally seen in terrestrial animals. We  
28 therefore find no mechanical support for a low CoT in these species. This study  
29 illustrates the need for research on a wider range of chelonians to discover whether  
30 there are indeed general trends in mechanical and metabolic energy costs.

31

32 **Introduction**

33

34 Chelonians have very limited body wall mobility due to their rigid or semi-rigid  
35 carapace and plastron. This unique body morphology has been studied in the context  
36 of swimming (Mayerl and Blob, 2017; Pace et al., 2001; Rivera and Blob, 2013; Rivera  
37 et al., 2006); bone loading (Butcher and Blob, 2008; Young and Blob, 2015; Young et  
38 al., 2017), pelvic morphology (Mayerl et al., 2016), shoulder girdle mobility (Schmidt  
39 et al., 2016), and self-righting behaviour (Várkonyi and Domokos, 2007), but terrestrial  
40 kinematic analyses are rare (Blob et al., 2007; Rivera and Blob, 2010; Schoenfuss et  
41 al., 2010). The metabolic CoT has been investigated during walking in only two  
42 species: *Emydura macquarii*, and *Terrapene ornata* (Zani and Kram, 2008). Both  
43 species showed CoT half of that expected but the data are noisy, with low  $R^2$ , and are  
44 curious given the likely influence of the mass and rigidity of the carapace-plastron  
45 structure of the body wall.

46

47 There are several possible explanations for this low metabolic CoT in chelonians. Their  
48 specialized articulation between the scapula and carapace (Nagashima et al., 2013),  
49 which eliminates the need for the 'muscular sling' present in other quadrupeds (Carrier  
50 et al., 2006), may save energy. In addition, some turtles possess mobile pectoral  
51 girdles which could also help with locomotor efficiency (Mayerl et al., 2019).  
52 Chelonians also possess slower, more efficient muscles (*in vitro*) relative to other  
53 vertebrates (Woledge, 1968) and move slowly, thus increasing the duration of foot-  
54 ground contact which may reduce energy cost (Kram and Taylor, 1990). However,  
55 fluctuations in the kinetic energy (KE) and gravitational potential energy (PE) of the  
56 centre of mass (COM) that are  $180^\circ$  out of phase and of equal amplitude are optimal  
57 for maximizing energy recovery and this may be impaired by slow speed (Cavagna et  
58 al., 1977). In giant Galápagos tortoises (*Geochelone elephantopus*), KE amplitude is  
59 only one third of that of PE during walking (Zani et al., 2005) and the fluctuation is  
60 random demonstrating little mechanical energy recovery (~30%, compared to values  
61 up to 65-70% in dogs (Griffin et al., 2004)). Even so, the mechanical CoT in  
62 *Geochelone* (~0.41 J kg<sup>-1</sup> m<sup>-1</sup>) was similar to other limbed animals (Zani et al., 2005).  
63 Unfortunately, the metabolic CoT has not been measured in *Geochelone* and the  
64 mechanical CoT has not been investigated in other chelonians.

65

66 We investigated body kinematics and energy recovery in three sympatric, fresh-water  
67 turtles: spiny soft-shell turtle, *Apalone spinifera*; common snapping turtle, *Chelydra*

68 *serpentina*; and red-eared slider, *Trachemys scripta*. These species are ecologically  
69 distinct: *Apalone* is a free-swimming aquatic form (Plummer et al., 1997) with reduced  
70 carapace structure; *Chelydra* is a bottom-dwelling aquatic form with a pronounced  
71 carapace and limited plastron but capable of extended terrestrial locomotion  
72 (Steyermark et al., 2008); *Trachemys* is semi-aquatic (Cagle, 1950) with a robust  
73 carapace and plastron. Based on findings in other species we would predict a low  
74 mechanical cost of locomotion in these taxa, but that costs might not depend on  
75 pendular energy recovery. We would also expect higher costs of locomotion in the  
76 more aquatic species since these are more likely to have anatomical specialisations  
77 for swimming.

78

## 79 **Materials and Methods**

80

81 6 *Apalone spinifera* and 13 *Trachemys scripta* were wild-collected near lake Lewisville  
82 TX and 21 *Chelydra serpentina* were sourced from captive stock held at the University  
83 of North Texas (UNT). All experiments were approved by the UNT. Turtles were  
84 maintained at 24°C in 50-500 L tanks. All species were fed Mazuri® food (Mazuri®,  
85 PMI Nutrition International, Brentwood, MO) 2-4 times per week and maintained on a  
86 12:12 h light-dark cycle. On the day of study, five infrared markers were attached to  
87 the shell using non-toxic cyanoacrylate (four on the perimeter and one to the apex).  
88 An Optitrack system ([www.optitrack.com](http://www.optitrack.com)) with six 0.3 megapixel, 100 fps FLEX:V100  
89 cameras was used for motion capture. The animals were placed on a 3x5 m floor mat  
90 to prevent slipping and recorded continuously for 5-10 minutes. Each animal was  
91 tested at least 5 times on separate days.

92

93 Bouts were identified from the kinematic data by finding periods where the animal  
94 followed an approximately straight course with constant speed over several gait cycles.  
95 139 bouts were analysed using a variant of Procrustes shape analysis that used the  
96 visible markers to calculate a mean shape by overlaying each set of markers and  
97 finding the translation and rotation that minimises the sum of square distances  
98 between the matched markers. We then calculated the translation and rotation that  
99 mapped the mean shape to the markers in an individual frame. The coordinate system  
100 for this mapping was chosen so that the origin was the centroid of the markers in the  
101 first frame, the Z axis was vertical and the X axis was oriented to the mean direction

102 of travel. A 10 Hz two-pole Butterworth low-pass filter was applied using Matlab filtfilt  
103 function (www.mathworks.com) to reduce the high frequency noise in the kinematic  
104 data (Winter, 1990). The translation represents the linear motion of the centroid of the  
105 marker system, approximating the centre of mass of the animal. The rotation  
106 represents the rotation of the animal's carapace during locomotion.

107

108 Energy recovery was calculated from the interchange between the gravitational and  
109 kinetic energy using the formulation shown in Equation 1 (Dipaola et al., 2016) where  
110  $ER$  is energy recovery (%),  $W_p$  is the difference between the maximum and minimum  
111 gravitational potential energy in a single stride,  $W_k$  is the difference between the  
112 maximum and minimum kinetic energy of the centre of mass, and  $W_{totCM}$  is the  
113 difference between the maximum and minimum values of the sum of kinetic and  
114 gravitational potential energy over the stride.

115

116 Equation 1

$$117 \quad ER = \frac{(W_p + W_k) - W_{totCM}}{(W_p + W_k)} \times 100$$

118

## 119 **Results**

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121 During recorded locomotor trials *Apalone* starts to move immediately and rapidly in a  
122 straight line. The animal can repeat this action but tires, with each repeat slowing and,  
123 eventually, remaining motionless with head and limbs retracted. Both *Chelydra* and  
124 *Trachemys* tend to remain immobile with their heads and limbs retracted when first  
125 placed on the substrate. After a few minutes they begin to walk slowly, often in a  
126 circular fashion. However, this can transition into a more directed, faster, straight line  
127 movement. Both *Chelydra* and *Trachemys* tend to pause when walking and do not  
128 appear to tire rapidly.

129

130 The speeds chosen by turtles suggest that there are no characteristic, preferred  
131 speeds for these species, and little obvious effect of body size (Figure 1A-D). Both  
132 *Apalone* and *Chelydra* are larger than *Trachemys* in our sample but only *Apalone* is  
133 noticeably faster than the others, both in absolute terms and when geometrically

134 corrected for body mass. However, the fastest animal recorded was one of the smaller  
135 *Trachemys* specimens. Rotations of the carapace were variable (Figure 1E) and there  
136 is no obvious effect of body mass but there are species level differences, with  
137 *Trachemys* having less rotation than the others. Yaw was very pronounced in *Chelydra*  
138 whereas in the other species roll is the largest component.

139

140 The calculated energy recovery is low (Figure 2ABC) as predicted from the variations  
141 in both the linear and angular kinematics. We also calculated the external work directly  
142 by summing the positive components of total energy (PE + KE) which provides a useful  
143 measure of the mechanical CoT (external work/distance). The mass-specific power  
144 (external work/duration) for the individual species at their different self-selected  
145 speeds (Figure 2D) shows the characteristic increase in power with speed that would  
146 be expected but there is a great deal of scatter, and the effect for *Apalone* is very weak.  
147 Figure 2E shows the effect of speed on the mass-specific mechanical CoT. Only  
148 *Chelydra* and *Trachemys* show a positive relationship between mechanical CoT and  
149 speed. Figures 2FG show the between-species mean values for power and  
150 mechanical CoT which are appreciably higher on average for *Apalone*.

151

## 152 **Discussion**

153

154 Our mechanical CoT data provide no evidence for the low metabolic CoT previously  
155 reported (Zani and Kram, 2008). The mean mechanical CoT was higher in each of  
156 these species compared to other legged animals (Full reports values around  $1 \text{ J kg}^{-1}$   
157  $\text{m}^{-1}$  for mammals, birds, crustacea and insects with no appreciable effect of body size  
158 (Full and Tu, 1991) but there is considerable variation from  $0.47 \text{ J kg}^{-1} \text{ m}^{-1}$  in  
159 *Spermophilus tereticaudus* to  $1.85 \text{ J kg}^{-1} \text{ m}^{-1}$  in *Macaca speciosa* (Nudds et al., 2009)).  
160 Being aquatic or semi-aquatic potentially represents a trade-off in locomotor efficiency  
161 between locomotor modes. Indeed *Apalone*, the most aquatic, has the highest mass-  
162 specific mechanical CoT. These data also suggest that *Trachemys*, being semi-  
163 aquatic, can employ more energy efficient gaits. The higher values may therefore  
164 represent the gaits chosen for reasons other than energy efficiency such as escape,  
165 exploration, and crypsis. There was a great deal of between bout variation in the  
166 measured CoT (range  $0.65\text{-}10.9 \text{ J kg}^{-1} \text{ m}^{-1}$ ) which would suggest that the study  
167 animals were not choosing to minimise CoT. The only other experimental values for

168 chelonians show similar between bout variation (range 0.1-1.0 J kg<sup>-1</sup> m<sup>-1</sup>) (Zani et al.,  
169 2005) also suggesting a great deal of flexibility in CoT values used.

170

171 Pendular energy recovery for these animals seems unlikely. They showed no obvious  
172 preferred speeds, carapace rotations were variable, and the KE and PE of the body  
173 COM did not fluctuate relative to one another in a predictable fashion. Clearly, more  
174 studies are needed to understand how the various mechanisms that might lead to  
175 reduced energy costs are interacting within chelonians. Indeed measurements of  
176 external work are a very limited proxy for the actual mechanical cost of locomotion and  
177 ideally, full inverse dynamic studies should be performed (Winter, 1990), coupled with  
178 specific models of muscle energy conversion (Sellers et al., 2003).

179

180 The results illustrate the kinematic features that may underlie the differences in  
181 locomotor efficiency between the three species. *Apalone* is much faster than the other  
182 two species suggesting that out of an aquatic setting this species employs an escape  
183 gait. The other two species show an increase in cost of locomotion with speed, but for  
184 *Apalone*, all speeds are energetically expensive. The trunk rotation data are interesting  
185 since these represent the movements of a rigid or semi-rigid box that may impede  
186 some of the compensatory movements of the limb girdles and the vertebral column  
187 that may reduce CoT in other tetrapods, even though recent work has shown that in  
188 some turtle species, girdle movement can be surprisingly large (Mayerl et al., 2019).  
189 There are few comparative studies of body rotation and the values reported are  
190 typically between 4° to 10° (Byström et al., 2009; Dunbar, 2004; Dunbar et al., 2008;  
191 Jayes and Alexander, 1980; Stokes et al., 1989). The mean values shown are not  
192 therefore especially extreme, however this hides the fact that for some normal walk  
193 sequences we see much higher values (~25°) particularly in roll and yaw suggesting  
194 that carapace rotation is an important component of CoT.

195

## 196 **Conclusion**

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198 These three species of aquatic and semi-aquatic turtles have high mechanical CoT  
199 and little or no opportunity for pendular energy recovery. Observed gaits were highly  
200 variable with qualitative differences. This work illustrates the relatively poor level of  
201 understanding that we currently have for low speed locomotion where traditional

202 energy recovery models are not applicable, and there is a need for more detailed  
203 analysis across more species.

204

## 205 **Acknowledgements**

206

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208

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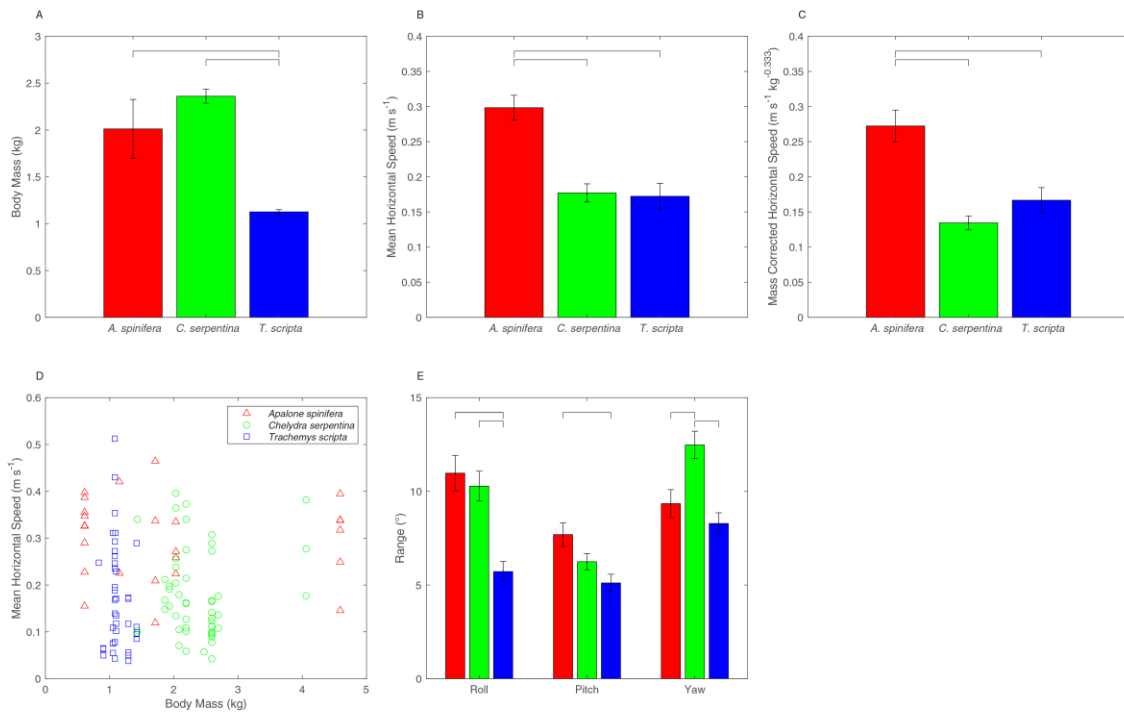
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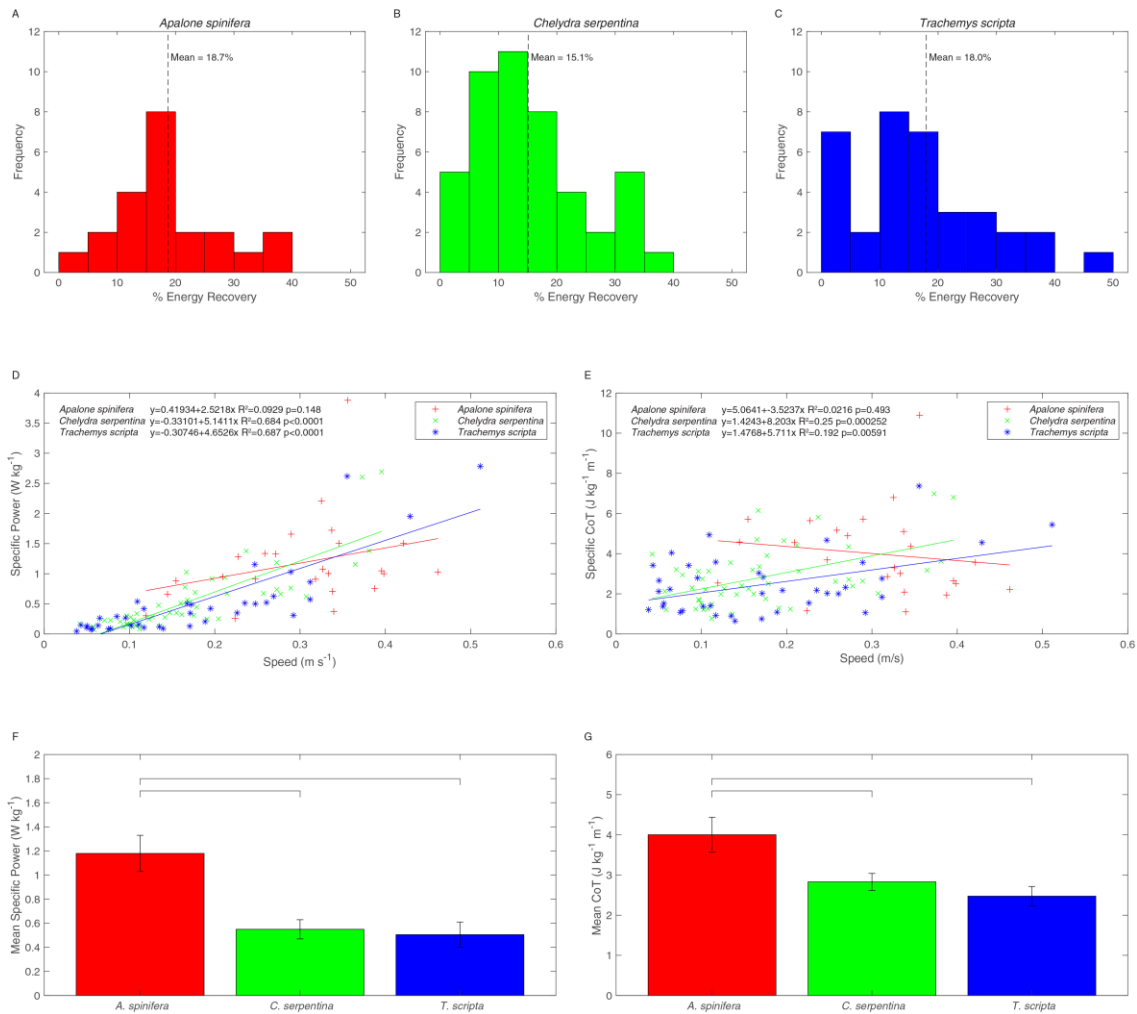
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307 Figure 1. (A) Mean body masses (one-way ANOVA  $F=25.983$ ,  $p<0.001$ ; Tukey HSD  
 308 AS:TS  $p<0.001$ , CS:TS  $p<0.001$ ). (B) Mean horizontal speeds (one-way ANOVA  
 309  $F=14.945$ ,  $p<0.001$ ; Tukey HSD AS:CS  $p<0.001$ , AS:TS  $p<0.001$ ). (C) Mass corrected  
 310 horizontal speeds (one-way ANOVA  $F=17.609$ ,  $p<0.001$ ; Tukey HSD AS:CS  $p<0.001$ ,  
 311 AS:TS  $p<0.001$ ). (D) Self-selected speeds recorded in the different locomotor bouts.  
 312 (E) Carapace rotation ranges (Roll: one-way ANOVA  $F=12.782$   $p<0.001$ ; Tukey HSD  
 313 AS:TS  $p<0.001$ , CS:TS  $p<0.001$ ; Pitch:  $F=5.453$   $p=0.005$ ; Tukey HSD AS:TS  $p=0.004$ ;  
 314 Yaw:  $F=10.723$   $p<0.001$ ; Tukey HSD AS:CS  $p=0.013$ , CS:TS  $p<0.001$ ). Error bars  
 315 show the standard errors of the mean. *Apalone*  $N=5$ , *Chelydra*  $N=10$ , *Trachemys*  $N=8$ .

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321 Figure 2. (A-C) Histograms showing the distributions of energy recovery measured for  
 322 the locomotor bouts. The vertical lines show the arithmetic means for each species.

323 (D) Mechanical power; (E) Mechanical CoT; (F) Mean power (one-way ANOVA

324  $F = 10.063$   $p < 0.001$ ; Tukey HSD AS:CS  $p < 0.001$ , AS:TS  $p < 0.001$ ); (G) Mechanical CoT

325 (one-way ANOVA  $F = 6.648$   $p = 0.002$ ; Tukey HSD AS:CS  $p = 0.014$ , AS:TS  $p = 0.002$ ).

326 Error bars show the standard errors of the mean.

327