

# Lung Ventilation During Treadmill Locomotion in a Semi-Aquatic Turtle, *Trachemys scripta*

TOBIAS LANDBERG<sup>1\*</sup>, JEFFREY D. MAILHOT<sup>2</sup>, AND ELIZABETH L. BRAINERD<sup>3</sup>

<sup>1</sup>*Ecology and Evolutionary Biology Department, University of Connecticut, Storrs, Connecticut*

<sup>2</sup>*UMASS Memorial Medical Center, Worcester, MA*

<sup>3</sup>*Ecology and Evolutionary Biology Department, Brown University, Providence, Rhode Island*

**ABSTRACT** It is reasonable to presume that locomotion should have a mechanical effect on breathing in turtles. The turtle shell is rigid, and when the limbs protract and retract, air in the lungs should be displaced. This expectation was met in a previous study of the green sea turtle, *Chelonia mydas*; breathing completely ceased during terrestrial locomotion (Jackson and Prange, 1979. *J Comp Physiol* 134:315–319). In contrast, another study found no direct effect of locomotion on ventilation in the terrestrial box turtle, *Terrapene carolina* (Landberg et al., 2003. *J Exp Biol* 206:3391–3404). In this study we measured lung ventilation during treadmill locomotion in a semi-aquatic turtle, the red-eared slider, *Trachemys scripta*. Sliders breathed almost continuously during locomotion and during brief pauses between locomotor bouts. Tidal volume was relatively small (~1 mL) during locomotion and approximately doubled during pauses. Minute ventilation was, however, not significantly smaller during locomotion because breath frequency was higher than that during the pauses. We found no consistent evidence for phase coupling between breathing and locomotion indicating that sliders do not use locomotor movements to drive breathing. We also found no evidence for a buccal-pump mechanism. Sliders, like box turtles, appear to use abdominal musculature to breathe during locomotion. Thus, locomotion affects lung ventilation differently in the three turtle species studied to date: the terrestrial *Te. carolina* shows no measurable effect of locomotion on ventilation; the semi-aquatic *Tr. scripta* breathes with smaller tidal volumes during locomotion; and the highly aquatic *C. mydas* stops breathing completely during terrestrial locomotion. *J. Exp. Zool.* 2008. © 2008 Wiley-Liss, Inc.

---

**How to cite this article:** Landberg T, Mailhot JD, Brainerd EL. 2008. Lung ventilation during treadmill locomotion in a semi-aquatic turtle, *Trachemys scripta*. *J. Exp. Zool.* 309A:[page range].

---

The body plan of turtles is unique in that the ribs have fused with dermal bone into a carapace and the gastralia and some pectoral girdle bones have been incorporated into the plastron (reviewed in Gilbert et al., 2008). The lungs, limb girdles, abdominal muscles, and other viscera are all contained together within the shell formed by these bony elements. Because the volume within the turtle shell is nearly constant, retraction of the pectoral or pelvic limb and girdle elements into the shell drives air out of the lungs, whereas protraction of limb elements creates subatmospheric pressures that can produce inhalation (Gans and Hughes, '67; Gaunt and Gans, '69). This constant volume constraint suggests that during locomotion,

limb movements should affect the mechanics of lung ventilation.

Experimental evidence from adult female green sea turtles (*Chelonia mydas*) suggests that locomotion may interfere with breathing performance (Prange and Jackson, '76; Jackson and Prange, '79). During terrestrial locomotion, *C. mydas* stops

---

Grant sponsor: US National Science Foundation; Grant numbers: 9875245; and 0316174.

\*Correspondence to: Tobias Landberg, Ecology and Evolutionary Biology Department, University of Connecticut, 75 North Eagleville Road, Storrs, CT 06269. E-mail: tobias.landberg@uconn.edu

Received 30 November 2007; Revised 28 May 2008; Accepted 12 June 2008

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jez.478

breathing during bouts of locomotion and resumes breathing during pauses in locomotion (Jackson and Prange, '79). Jackson and Prange ('79) suggested that the use of limb musculature for both locomotion and breathing could prevent the two behaviors from being performed at the same time.

In contrast, a study of breathing and locomotion in a terrestrial turtle, *Terrapene carolina*, found that box turtles breathe continuously during locomotion and there is no measurable effect of the stride cycle on lung ventilation (Landberg et al., 2003). This remarkable result indicates that box turtles can decouple or minimize any effects the limbs have on body cavity pressure, most likely by the action of their abdominal muscles.

Two abdominal muscles, the transverse abdominis (TA) and the oblique abdominis (OA), are considered to be the primary ventilation muscles in turtles because they are present in all species studied to date (George and Shaw, '59; Shaw, '62) and they are consistently found to be active during lung ventilation (Gans and Hughes, '67; Gaunt and Gans, '69). These antagonistic muscles alternate bilateral muscle activity to produce exhalation (TA) and inhalation (OA) in turtles at rest (McCutcheon, '43; Gans and Hughes, '67; Gaunt and Gans, '69). The OA is a thin, cup-shaped muscle that lies just deep to the skin and spans the inguinal limb pockets anterior to each hindlimb between the carapace and the plastron. At rest, this muscle curves into the body cavity and has an action similar to the mammalian diaphragm. As it contracts, it flattens to move the flank postero-ventero-laterally, thereby reducing intrapulmonary pressure and driving inhalation when the glottis is open.

The TA lies deep to the OA. It originates along the inside of the carapace and is cupped around the posterior half of each lung. The TA raises intrapulmonary pressure as it contracts, producing exhalation when the glottis is open. The convex sides of the TA and the OA face each other so that when one muscle contracts and flattens, the other is stretched into a curved position from which it can contract to reverse the motion. The TA may act together with a diaphragmaticus muscle that wraps around the front half of the lungs (Gaunt and Gans, '69). The diaphragmaticus is often largest in highly aquatic turtle species such as softshelled turtles and absent in terrestrial turtles such as tortoises and box turtles suggesting a primary role in buoyancy control (Shaw, '62).

We chose *Trachemys scripta* for this study because the other two turtle species studied to date have a number of habitat specializations that may have influenced their breathing and/or locomotor behavior. In contrast, *Tr. scripta* retains a very typical semi-aquatic lifestyle that is common to most members of the family Emydidae. The lack of obvious morphological specializations of this species and its abundance in the wild and pet trade have made it the most common turtle used in laboratory studies.

The goals of this study were to determine if sliders breathe during locomotion and, if so, do they: (1) use a buccal force pump; (2) use respiratory-locomotor coupling; (3) use a limb pump; (4) show any effects of the stride cycle on the magnitude of breaths; (5) show any signs of ventilatory constraints imposed by locomotion.

## MATERIALS AND METHODS

### *Experimental animals*

Five subadult *Tr. scripta elegans* (Weid) were housed together in a 170-L aquarium where they had room to swim and bask under light (14:10 light cycle). Masses varied daily but ranged between about 225 and 300 g. These animals were kept at a water temperature of  $26 \pm 3^\circ\text{C}$  and fed crickets, worms, fish, and Reptomin (Melle, Germany). Individuals were identified by a notch filed into a different marginal scute. All experimental procedures and animal rearing protocols were approved and performed in accordance with the University of Massachusetts Amherst Institutional Animal Care and Use Committee.

### *Airflow measurements*

To measure ventilation in *Tr. scripta* during locomotion, a custom mask was constructed for each turtle that covered both mouth and nares. These pneumotach masks were almost identical to those developed for studying ventilation in *Te. carolina* (Landberg et al., 2003).

The masks were made of high-viscosity rubber-based dental impression material (Henry Schein Company, Melville, NY) and custom-fit to the head of each turtle. During each of the several stages in the mask-building process, individual turtles wore a padded restraint collar that prevented them from pulling their heads back into the shell. During the first stage, a small amount ( $\sim 0.2\text{ cm}^3$ ) of modeling clay was applied over the nares of the turtle, which created an air-filled space in the

mask when it was removed at later stages. The mouth was uncovered during this stage allowing animals to breathe through the mouth. Dental impression material was applied over the clay and the head (except for the lower jaw and eyes). Once set, the mask was removed from the turtle and allowed to cure overnight. The mask was then trimmed, the clay was removed from inside, and a breathing port was glued into the front of the mask (in front of the nares where the clay had been). The breathing port (~0.5-cm section of a Bic Round Stic pen, Shelton, CT; 0.8 cm outer diameter and 0.6 cm inner diameter) was sealed and glued to the mask with cyanoacrylate. In stage two of mask construction, the mouth was covered by the mask and turtle breathed via the nares through the open breathing port. A thin bead of clay was placed over the area where the upper and lower beaks meet and another bead ran along the midline of the upper beak connecting to the breathing port. Freshly mixed dental impression material was applied over the clay and the entire head except the nares. The first, previously cured portion of the mask with the breathing port was then pressed onto the wet dental impression material. After the composite mask set, it was removed, allowed to cure overnight, and trimmed. When the clay was removed, the airspace in the mask (~0.3 cm<sup>3</sup>) allowed the turtle to breathe through either the nares or the mouth. The finished masks weighed less than 3 g. On the day of the experiment, the mask was glued to the turtle's head with surgical adhesive (cyanoacrylate) and the pneumotach was inserted into the port and sealed with a small amount of petroleum jelly. The entire mask was sealed airtight around the nares and mouth channeling all ventilatory airflow through the breathing port.

The pneumotach was made from two 1-cm long sections of a 1-cm<sup>3</sup> syringe that were separated by a small piece of 53 μm screen. Five millimeter pieces of a 18 gauge needle were inserted on either side of the screen. These needle segments connected 160pe tubing to the differential pressure transducer (Validyne DP103-06, Northridge, CA). The pneumotach was calibrated with known airflow rates and by injecting known volumes of air via syringes. The voltage signal from the pressure transducer was sent through a carrier demodulator (CD-15) to a computer, which displayed a trace in Superscope II 2.1. Exhalation and inhalation volumes were measured as the area between the zero line and the trace above or below zero, respectively.

In order to allow subsequent frame-by-frame analysis, a live feed of the breathing trace and a standard dorsal-view video of the animal (30 frames/sec) were synchronized and recorded onto video tape. The image was split so that the bottom half of the screen showed the turtle locomoting on the treadmill, whereas the top half of the screen showed the breathing trace.

Experiments were run on a low-speed custom-built treadmill (surrounded with clear acrylic walls to keep the turtles on the treadmill). Cloacal temperatures were monitored every hour during the experiment and maintained as close as possible to 30°C. A small space heater placed at one end of the treadmill helped control temperature. On occasion, the animal rested near this heat source resulting in a rapid rise in cloacal temperature. These increases (2–6°C) could be rapidly reversed and were most likely not representative of core body temperature.

The experiments were separated into four segments: acclimation, pre-exercise, locomotion, and recovery. Before acclimation, the mask was glued on to the animal and the animal was placed into the treadmill chamber. Acclimation lasted 1 hr. During the second hour, pre-exercise ventilation was measured. An experimenter was present and visible during the entire experiment. This seemed to deter the animals from wandering around the treadmill chamber during pre-exercise, pause, and recovery. Locomotion followed pre-exercise and was variable in duration. The goal was to record at least ten bouts of locomotion each containing at least ten breaths and ten strides per individual. For some experiments, this goal was never reached and these experiments were discarded. For the data reported in this study it took 1–3 hr to obtain ten satisfactory bouts. The animals most often initiated locomotor bouts themselves, but sometimes they were stimulated to locomote by starting the treadmill and/or rolling the treadmill back until the shell gently tapped against the acrylic wall. Treadmill speed was manually controlled to match each turtle's voluntary locomotor speed. The final hour of the experiment recorded post-exercise recovery ventilation, and the data recorded in the first 20 min immediately after locomotion were used for recovery analysis. The masks were removed after the experiment and the animals were returned to their aquarium.

### *Data analysis*

Twenty-minute segments during pre-exercise, locomotion, and recovery were analyzed from four

individuals (one experimental animal had an incomplete data set and was excluded from tidal volume, breath frequency, and minute volume analyses). Pre-exercise segments were selected to minimize the animals' movement in the chamber, which was usually the last 20 min of the pre-exercise hour. The 20-minute segments of locomotion were chosen to maximize the amount of time spent actually locomoting (the remainder of the time consisted of pausing between locomotor bouts). Each breath during the 20-min period of locomotion was designated as occurring either during a locomotor bout or during a pause between bouts and these breaths were analyzed separately. Lastly, the first 20-min segment (immediately after locomotion) of recovery was chosen to best represent post-exercise recovery. This created four experimental "behaviors" to be analyzed (pre-exercise, locomotion, pause, and recovery).

The tidal volume of every breath occurring during the four behaviors was measured individually. All tidal volume values from the four behaviors of four individuals were run in a two-way analysis of variance (ANOVA, StatView 4.5) to test for the effects of the behavior and individual. Post hoc tests, Fisher's protected least significant difference (Fisher's PLSD), were used to test for significant differences in pairwise comparisons between the four behaviors after significant *F*-values were observed for behavior in the ANOVA.

Average minute volume (mL/min) was calculated by summing up the tidal volumes in each experimental segment and dividing this sum by the duration of the segment (20 min for pre-exercise and recovery and variable amounts of time for locomotion and pause depending on the individual animal's behavior). This was done for all four behaviors in each of the four individuals, resulting in 16 average minute volumes. Average breath frequency (breaths/min) was calculated similarly by counting the number of breaths during each segment and dividing that number by the segment duration. Average minute volume and breath frequency were obtained to compare relative differences in minute volume and breath frequency between the four behaviors. As the absolute value of minute volume and breath frequency varied between individuals, paired *T*-tests (StatView 4.5) were used for pairwise comparisons between the four behaviors (values paired by individual).

Phase analysis can reveal the relationship of breaths to the stride cycle on a polar graph. The

polar graph is a circle that represents the stride cycle from 0° (start of stride) to 360° (return to original starting position). Peak airflow from individual breaths was plotted according to the time of occurrence during the stride cycle of which it occurred. This allowed us to test if the breaths were distributed randomly through the stride cycle or were clustered or absent at any point in the stride cycle. For each of the five individuals, the first ten locomotor bouts containing both ten strides and ten breaths were used in this analysis (these all fell within the same 20-min segments used for tidal volume analysis). A kinematically distinct point in the stride cycle, maximum right hindlimb extension (MHE), was chosen to anchor the polar plots (0°). MHE was defined as the video frame where the greatest knee and ankle extension occurred. Timing of MHE (0.0625 sec was the time interval between frames) was measured from the video recordings, whereas peak ventilatory airflow was measured from the Superscope file (sampled at least at 200 Hz). The timing of peak airflow could be compared with the relative timing of the stride cycle through the equation: Relative timing of breath peak = ((actual time of breath peak - actual time of MHE)/stride duration). The total stride duration was calculated by subtracting the MHE preceding the breath peak from the MHE succeeding the breath peak. The result of this equation was a number between 1 and 0 that, when multiplied by 360, could be plotted on a polar graph as a degree measure. All inhalations and exhalations from each individual (range = 225-259 breaths per individual) were pooled and analyzed separately using Raleigh's test of circular uniformity (Zar, '96). Polar graphs were created for each of the ten bouts of locomotion per individual showing not only the phase relationship of breaths to the stride cycle (around the circle) but also the magnitude of each tidal volume (on the radius).

The relationship between average breathing frequency and average stride frequency per bout was analyzed using analysis of covariance (ANCOVA) with individual turtle as a factor and stride frequency as a continuous covariate.

## RESULTS

Average locomotor speed on the treadmill varied from 0.075 m/sec to almost 0.18 m/sec (Fig. 1). Both stride length and stride frequency contributed to increases in speed, but stride length

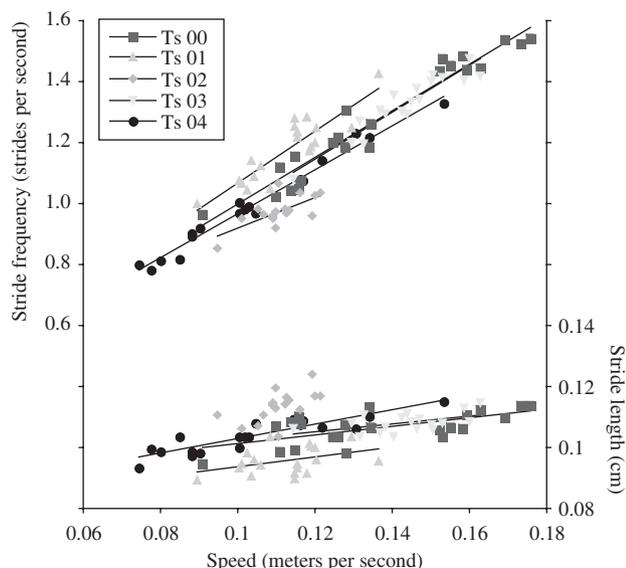


Fig. 1. Stride frequency and stride length vs. treadmill speed during locomotion in *Trachemys scripta*. Each point represents the average stride frequency or the average stride length during a bout of treadmill locomotion (at least ten strides per bout). All regressions are significant with the exception of stride length in Ts01 (see Tables 1 and 2 for regression statistics).

TABLE 1. Regression results for stride frequency vs. speed

Individual	Slope	Intercept	$R^2$	$P$ -value
Ts00	7.68	0.23	0.94	<0.0001
Ts01	8.52	0.22	0.79	<0.0001
Ts02	5.04	0.42	0.48	0.0021
Ts03	7.67	0.23	0.89	<0.0001
Ts04	7.17	0.25	0.98	<0.0001

increases were small relative to the increases in stride frequency (Tables 1 and 2). The gait used during these trials was typical for turtles, a diagonal couplet with duty factors for each limb around 75% of the stride cycle (Fig. 2).

In agreement with previous studies (e.g. Vitalis and Milsom, '86a), we found that *Tr. scripta* breathes intermittently when at rest, generally in bouts of six to ten breaths interspersed with variable periods of apnea. During treadmill locomotion, however, pneumotach flow traces show that *Tr. scripta* breathes continually with almost no apneic periods (Fig. 3).

We observed some nonventilatory buccal oscillations at rest, but found no evidence that gular pumping contributes to lung ventilation at rest or during locomotion in *Tr. scripta*. In pneumotach flow traces, gular pumping would create a

TABLE 2. Regression equations for stride length vs. speed

Individual	Slope	Intercept	$R^2$	$P$ -value
Ts00	0.14	0.09	0.04	0.0014
Ts01	0.16	0.08	0.17	0.0774
Ts02	0.44	0.06	0.36	0.0111
Ts03	0.13	0.09	0.25	0.0333
Ts04	0.24	0.08	0.79	<0.0001

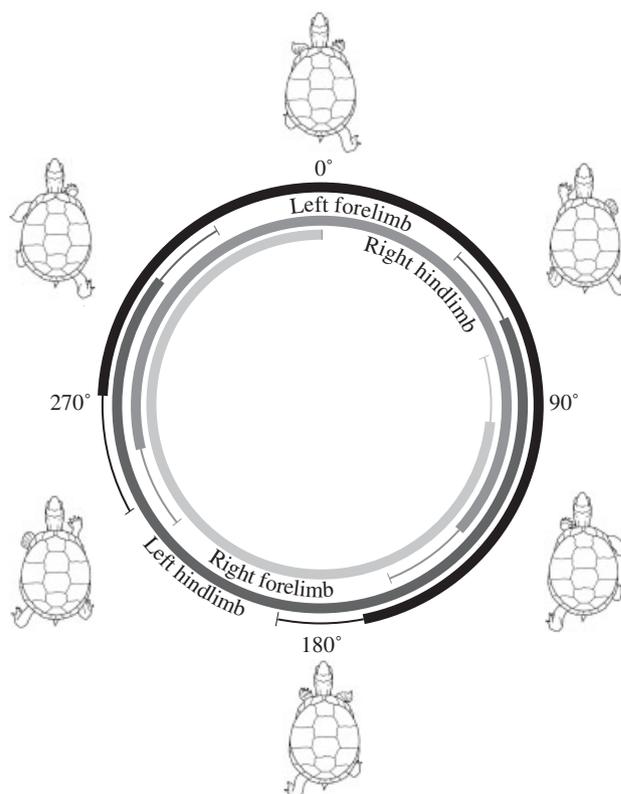


Fig. 2. Polar graph of footfall patterns during treadmill locomotion. Each bar shows the average contact time (+ standard deviation) of one limb. The end of right hindlimb contact ( $0^\circ$ ) shows no error because that point was defined as the beginning of every stride cycle. This representative graph was constructed from the two locomotor bouts shown in Figure 3.

distinctive pattern of several small inspirations followed by a large exhalation (Brainerd and Owerkowitz, 2006).

Breath frequency did not increase or decrease consistently with increasing stride frequency (ANCOVA,  $P = 0.99$ ; Fig. 4). Average breath frequency differed among individuals (ANCOVA,  $P < 0.0001$ ; Fig. 4); however, there was no individual by stride frequency interaction (ANCOVA,  $P = 0.23$ ) indicating that all individuals had a similar independence of breathing and stride frequencies.

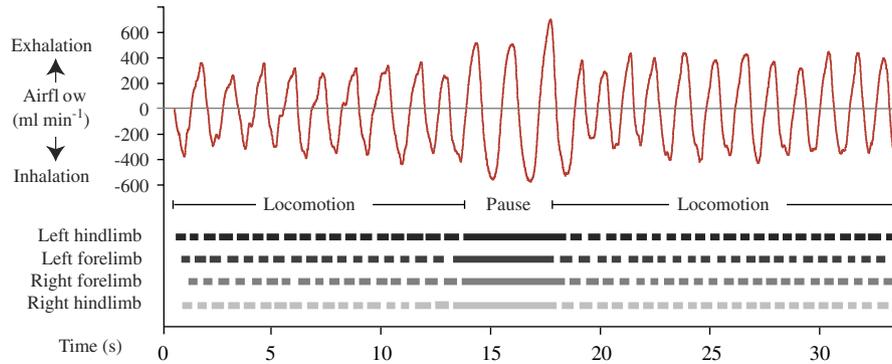


Fig. 3. Representative airflow trace from individual 01 (above in red) and the footfall pattern (below in shades of gray) during two bouts of locomotion separated by a short pause. Contact times for each limb are shown as solid bars. Note that breathing is continuous throughout the sequence but the breaths are slightly larger during the pause than during the locomotor bouts.

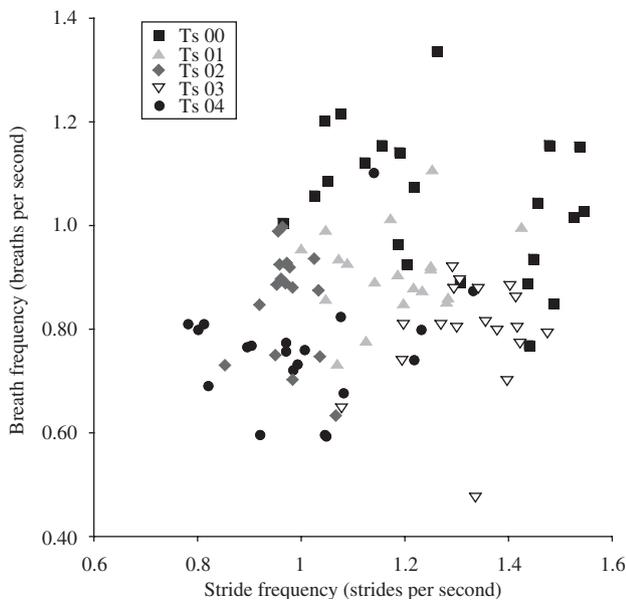


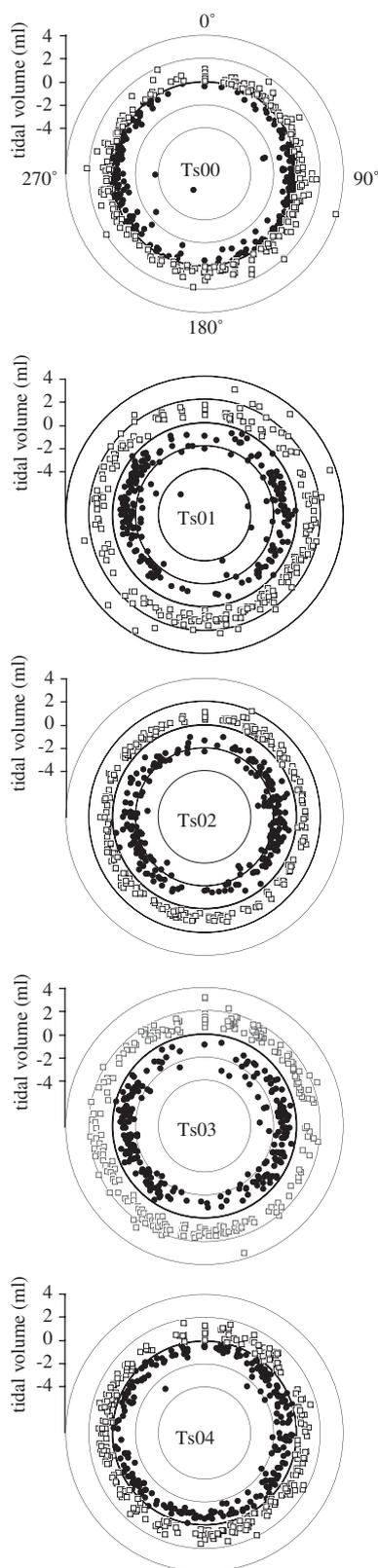
Fig. 4. Breath frequency vs. stride frequency during treadmill locomotion in five *Trachemys* individuals. Each point represents the average breath frequency of a treadmill locomotor bout plotted against the average stride frequency of that same bout (at least ten strides per bout). Average breath frequency is not affected by stride frequency (ANCOVA,  $P > 0.05$ ; see Results for further details). ANCOVA, analysis of covariance.

Plotting the phase of peak inhalatory and exhalatory airflow relative to the  $360^\circ$  stride cycle reveals no consistent relationship between footfall and breathing patterns in *Tr. scripta* (Fig. 5). In four of the five individuals there was no statistically significant phase relationship between the stride cycle and the timing of peak airflow ( $P > 0.05$ , Raleigh's test), but individual Ts01 showed a nonrandom distribution of breaths

relative to the stride cycle (both inhalations and exhalations were nonuniformly distributed;  $P < 0.01$ , Raleigh's test). Because the gait used by these turtles is symmetrical, we also tested for bimodal clustering of breaths by axially transforming the angular data (Batschelet, '81). Subsequent Raleigh's tests were also nonsignificant, indicating uniform distributions and a lack of bimodality for all individuals ( $P > 0.05$ ).

Breath magnitude might still be affected by the stride cycle despite breaths being uniformly distributed around the stride cycle. We examined the magnitude of breaths from all five individuals visually by plotting both peak air flow rates and tidal volume as functions of the stride cycle. We found no observable, consistent effect of the stride cycle on either measure of breath magnitude (Fig. 5, distance of the points from the bold zero line indicates the magnitude of tidal volume).

Tidal volumes measured in this study were generally small, averaging just over 1 mL during locomotion and doubling to over 2 mL during the brief pauses between locomotor bouts (Figs. 3 and 6). Using two-way ANOVA with tidal volume as the dependent variable and individual and behavior as factors, we found significant effects of individual and behavior. Individuals Ts00, Ts01, Ts03, and Ts04 were different from each other at the  $P < 0.0001$  level (except Ts01 and Ts04 were not different from each other  $P > 0.05$ ; Fisher's PLSD post hoc tests). Pre-exercise tidal volumes were significantly different from locomotion, pause, and recovery ( $P = 0.0314$ ,  $P < 0.0001$ , and  $P < 0.0013$ , respectively; Fisher's PLSD post hoc tests). Locomotion differed significantly from pauses between locomotor bouts but not from recovery ( $P < 0.0001$  and  $P > 0.05$ , respectively;



Fisher's PLSD post hoc tests). Recovery and pause also differed significantly ( $P < 0.0001$ ; Fisher's PLSD post hoc tests).

Average breath frequency ranged from 6 breaths per minute during pre-exercise to 46 breaths per minute during locomotion (Fig. 6). Breath frequency was higher during locomotion than during pre-exercise, pause, and recovery ( $P = 0.0023$ ,  $P = 0.0349$ , and  $P = 0.0204$ , respectively;  $T$ -test paired by individual). Pre-exercise and pause also differed significantly ( $P = 0.0022$ ;  $T$ -test paired by individual). Recovery values did not differ statistically from pre-exercise or pause ( $P > 0.05$ ;  $T$ -test paired by individual).

Average minute volumes ranged from 8 mL/min during pre-exercise to 50 mL/min during locomotion and 78 mL/min during brief pauses in locomotion (Fig. 6). Minute volumes during pre-exercise were significantly different from locomotion and pause values ( $P = 0.0086$  and  $P = 0.0321$ , respectively;  $T$ -test paired by individual). Locomotion and pause values did not differ significantly ( $P > 0.05$ ;  $T$ -test paired by individual). Recovery values did not differ from any of the other behaviors ( $P > 0.05$ ;  $T$ -test paired by individual).

## DISCUSSION

In the words of Mitchell and Morehouse (1863), "That the locomotive movements may, and perhaps do at times modify the respiratory process, may be taken for granted." Locomotion is expected to affect lung ventilation in turtles because both limb girdles flank the lungs and cyclical movement of the limbs impinges on the space occupied by the lungs. At rest, small bilaterally symmetrical limb movements are sufficient to drive both inhalation (during limb protraction) and exhalation (during retraction) in many species (Mitchell and Morehouse, 1863; McCutcheon, '43; Gans and Hughes, '67). In green sea turtles,

Fig. 5. Polar graphs of tidal volume vs. locomotor phase for five individual *Trachemys scripta*. Each graph shows the magnitude of inhalation (black circles, recorded as negative volumes) and exhalation (white squares, recorded as positive volumes) on the radius. The position of each breath around the circle shows the time of peak airflow from that breath relative to the stride cycle. Breaths were uniformly distributed with respect to the stride cycle for all turtles except Ts01 (see Results) indicating that there is not a fixed phase relationship between locomotion and breathing. A small number of exceptionally large breaths were cropped from these graphs to improve clarity.

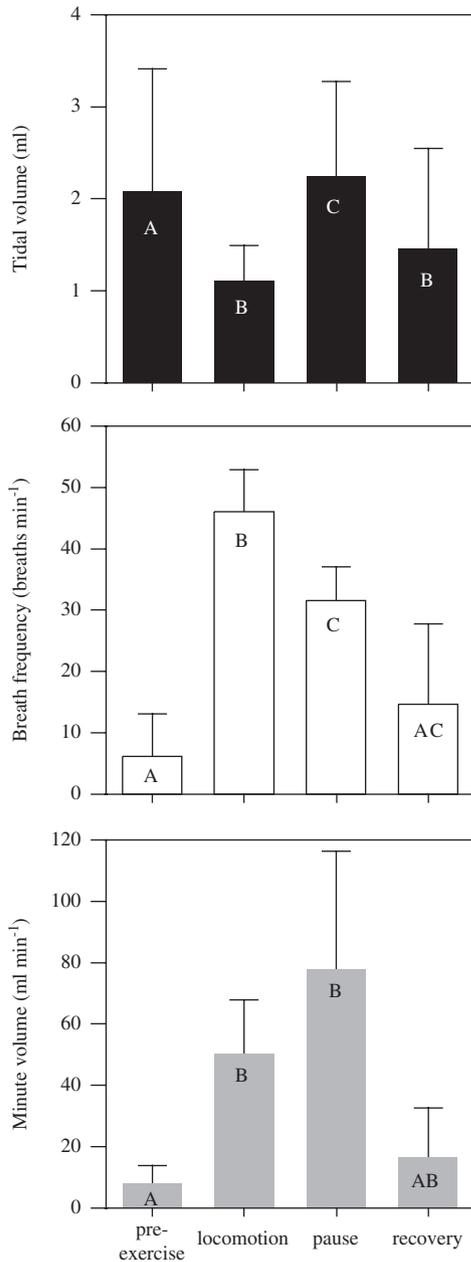


Fig. 6. Ventilation in *Trachemys* during four behaviors ( $n =$  four individuals). (A) Tidal volume (mean+SD). Two-way ANOVA with tidal volume as dependent variable and individual and behavior as factors with Fisher's PLSD post hoc tests reveals that all pairwise comparisons are significantly different except locomotion and recovery (see Results; bars sharing a letter are not significantly different). (B) Breath frequency (mean+SD). Locomotion is significantly different from all other behaviors and pause and pre-exercise are also different ( $T$ -test paired by individual; see Results). (C) Minute volume (mean+SD). Pre-exercise differs significantly from locomotion and pause ( $T$ -test paired by individual; see Results). ANOVA, analysis of variance; PLSD, protected least significant difference.

*C. mydas*, terrestrial locomotion interrupts breathing entirely (Jackson and Prange, '79). These large, almost entirely aquatic marine turtles crutch along the beach during the nesting period without ventilating the lungs and only breathe during the pauses between locomotor bouts. In contrast to *C. mydas*, box turtles (*Te. carolina*) breathe continually during treadmill locomotion without any measurable effect of the stride cycle on ventilation (Landberg et al., 2003). We chose *Tr. scripta* for this study because it has a semi-aquatic lifestyle that is typical for turtles and *Tr. scripta* is closely related to *Te. carolina* but lacks the morphological specializations associated with terrestriality (e.g. dome-shaped shell, very large lungs, and plastral hinge).

Our results indicate that *Tr. scripta* breathes almost continuously during treadmill locomotion using relatively small, rapid breaths (Fig. 3), and minute ventilation increases substantially during locomotion (Fig. 6). This pattern is similar to that of *Te. carolina* (Landberg et al., 2003) but differs markedly from the complete apnea exhibited by *C. mydas* during locomotion. This continuous breathing pattern is also distinct from the typical ventilatory pattern seen in resting turtles that is characterized by relatively long periods of apnea followed by short breathing bouts containing multiple breaths (e.g. Jackson, '71).

The results of this study also indicate no phase coupling between respiration and locomotion in *Tr. scripta*. Only one of the five sliders showed a nonrandom distribution of breaths relative to the stride cycle, and this individual (Ts01) exhibited both inhalations and exhalations around the entire stride cycle (but with some clumping) (Fig. 5). We also found no measurable effect of locomotion on breathing patterns in our previous study of the more fully terrestrial box turtle, *Te. carolina* (Landberg et al., 2003). The independence of breathing and locomotor cycles seen in *Te. carolina* therefore does not appear to be a specialization associated with a terrestrial mode of life, but rather is a more general pattern shared with *Tr. scripta*, a member of the Emydidae, retaining the more typical semi-aquatic lifestyle of the family.

Given the expected mechanical effects of limb and girdle motions on lung volume in turtles, it seems almost unthinkable that locomotor movements could be completely decoupled from breathing, yet this is what we find now for both *Tr. scripta* and *Te. carolina*. How it is possible that the pattern of limb movements can have no measurable effect on lung ventilation?

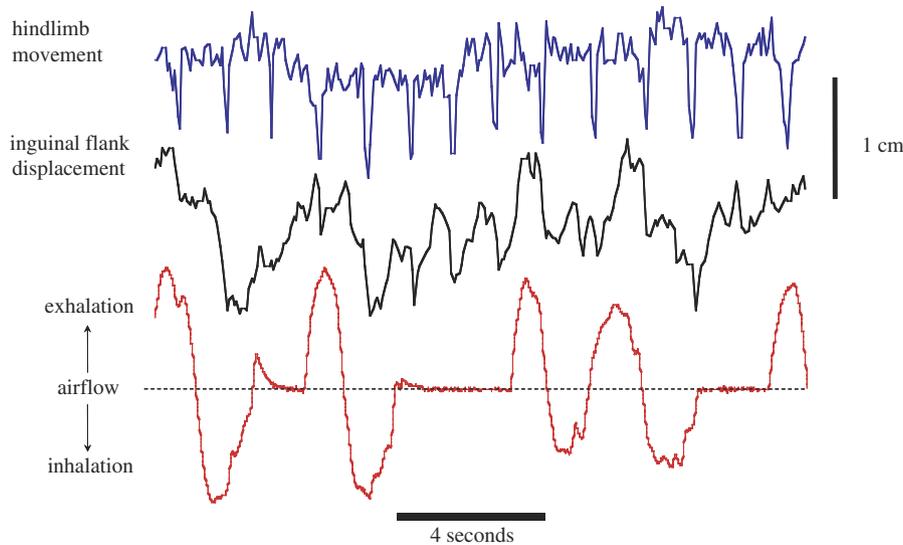


Fig. 7. X-ray kinematics of the hindlimb and inguinal flank with simultaneously recorded ventilatory airflow in *Terrapene carolina*. Each spike in the hindlimb trace (top) corresponds to lifting of the right hindfoot during treadmill locomotion. A metal marker was glued superficial to the oblique abdominis muscle on the inguinal flank and tracked from a digital X-ray video (middle trace). Airflow was measured from synchronized pneumotach mask (bottom trace). The inguinal flank reflects both the patterns of the airflow trace as well as the limb kinematics consistent with the hypothesis that a hybrid (left–right alternating and bilaterally symmetrical) motor pattern is driving the movements of the abdominal muscles. Methods are described in detail in Landberg et al. (2003).

One possible explanation could be that turtles breathe with a buccal pump during locomotion, thereby decoupling lung ventilation from appendicular movements. However, no evidence of a buccal pump was found in this study. Buccal pumping behavior would have produced airflow traces with multiple inhalations followed by a large single exhalation (e.g. Fig. 8 of Brainerd and Owerkowicz, 2006). Buccal pumping would also have been evident in the lateral-view video recordings as expansion–contraction cycles of the buccal cavity, but again no such evidence was found. Many instances of buccal oscillation were observed in *Tr. scripta* at rest, but as has been concluded in every previous study of turtle breathing (contra Agassiz, 1857), these oscillations were ventilating the buccal cavity and not the lungs (Mitchell and Morehouse, 1863; McCutcheon, '43; Druzisky and Brainerd, 2001; Landberg et al., 2003). We conclude that the ventilation mechanism used during locomotion in *Tr. scripta* does not involve the use of a buccal pump.

Another possible explanation for the complete decoupling of respiratory and locomotor cycles could be that the respiratory muscles are actively countering changes in pressure or volume induced by limb movements. Ventilation during terrestrial locomotion in *Tr. scripta* is almost certainly produced by the action of the abdominal mus-

cles—the OA for inspiration and the TA for exhalation. Electromyography demonstrates activity in these muscles during ventilation at rest and during aquatic locomotion in *Tr. scripta* (Currie, 2001, 2003), but electromyographic recordings of these muscles during terrestrial locomotion are yet to be made. Videofluoroscopy of breathing during locomotion in *Te. carolina* shows movements consistent with OA and TA activity during terrestrial locomotion (Landberg et al., 2003), providing some additional evidence that these muscles also are responsible for ventilation during terrestrial locomotion in *Tr. scripta*.

Evidence that the actions of the OA and the TA could be coordinated with locomotor movements comes from Currie's (2003) findings that the respiratory and locomotor motor patterns blend together during simultaneous breathing and swimming. Unilateral respiratory muscle activation coupled to hindlimb movement was superimposed over, or blended with, a bilateral motor pattern that coincided with breathing. The independence of stride cycle and breathing that we find in emydid turtles could be explained by such hybrid motor patterns if a bilateral breathing motor pattern is driven by a central pattern generator that is independent from a unilateral breathing pattern synchronized with the stride cycle. As long as the central pattern generators for

the unilateral and bilateral activities were independent and these independently controlled stimuli did not interfere, the muscle could sum the signals and could conceivably minimize effects of the limbs while driving ventilation. Evidence for such a motor pattern blending comes largely from turtles (e.g. Stein et al., '86; Earhart and Stein, 2000), but a variety of vertebrates show the type of modularity necessary to hybridize or blend functions (reviewed in Tresch et al., 2002). We have some kinematic evidence from *Te. carolina* that is consistent with this explanation. We tracked the inguinal flank (just superficial to the OA) of *Te. carolina* with a small metal marker in an X-ray videography experiment (Fig. 7). The motions of the flank reflect aspects of both airflow and limb kinematics indicating that the flanks may be moving under the influence of the hybrid alternating and bilateral motor pattern. Although these results are suggestive, the substantial pitch, roll, and yaw that the animals undergo during locomotion could not be accounted for in this analysis.

### ***Effects of locomotion on tidal volume and breath frequency***

Although we found no direct effect of limb movements on the breathing cycle, we did find evidence that locomotion may constrain tidal volume in a more global manner in *Tr. scripta*. Compared with pre-exercise, tidal volumes were small during locomotion, but increased to approximately twice the average locomotion values during brief pauses in locomotion (Fig. 6). We also found a significant decrease in breath frequency between locomotion and pause. The increase in tidal volumes and decrease in breath frequency canceled each other out so that the minute volumes between locomotion and pause were not significantly different. Overall, breath frequencies during both locomotion and pause fell within the lowest part of breathing work curves for *Tr. scripta* (Vitalis and Milsom, '86a,b), indicating that the animals chose combinations of frequencies and tidal volumes that minimized work.

Despite tidal volume being about only about 1 mL in *Tr. scripta* during locomotion, this volume is still sufficient to reach the gas exchange portions of the lung. The dead space in *Tr. scripta* is 0.6 mL/kg (Crawford et al., '76), which for our 0.2–0.3 kg animals would correspond to about 0.15–0.2 mL of dead space. Tidal volume goes up to almost 2 mL during brief pauses in locomotion.

Minute ventilation remains the same as during locomotion, because frequency goes down, but alveolar ventilation will increase in these pauses owing to the increase in tidal volume relative to the constant dead space.

We do not have good evidence to suggest why tidal volume is lower during locomotion than during pauses in *Tr. scripta*. It is possible that locomotion induces some general mechanical constraint, such as an overall increase in body cavity pressure that would oppose inhalation. The blended unilateral and bilateral motor pattern hypothesized above could raise body cavity pressure over resting values. Increased abdominal pressure during locomotion has been recorded in lizards and a crocodilian (Farmer and Hicks, 2000; Kidd and Brainerd, 2000; Munns et al., 2005). It is also possible that the neural control of breathing is affected by locomotion in a manner that decreases tidal volume, either with or without some simultaneous mechanical constraint.

Unlike our current findings for *Tr. scripta*, our previous results showed no difference in tidal volume between periods of locomotion and brief pauses in *Te. carolina* (Landberg et al., 2003). Therefore, the relationship between ventilation and terrestrial locomotion for the three turtle species studied to date is different for all three: the fully terrestrial box turtle, *Te. carolina*, shows no measurable effect of locomotion on ventilation at all; the semi-aquatic red-eared slider, *Tr. scripta*, shows no phase effect of ventilation, but overall tidal volume is lower during locomotion; and breathing stops completely during terrestrial locomotion in the green sea turtle *C. mydas*.

### ***Evolution of lung ventilation mechanisms in turtles***

We have documented effective lung ventilation during terrestrial locomotion in *Te. carolina* and *Tr. scripta*, and we have observed breathing movements during terrestrial locomotion in *Pelomedusa subrufa*, *Chelydra serpentina*, and *Gopherus polyphemus*. On the basis of these findings, we hypothesize that the ability to breathe during terrestrial locomotion is primitively shared by all turtles and secondarily lost in adult *C. mydas*. We predict that juvenile cheloniid sea turtles that unlike the adults use a typical turtle gait are able to ventilate their lungs during terrestrial locomotion. Additionally, the adults and juveniles of leatherback sea turtles, *Dermochelys coriacea*, both use a bilateral crutching gait

on land. Comparison between leatherback and green sea turtles could provide useful data on the effects of locomotor mode on breathing mechanics.

One of the defining features of turtles is that their ribs are fused into the carapace, thereby making costal aspiration, which is the primitive amniote breathing mechanism, impossible (Brainerd and Owerkowicz, 2006). Some researchers have implied that the carapace came first, "...one can readily see that the immobility of the ribs and the rigidity of the shell called for new ways of aerating the lungs. This difficult problem has given inquisitive mankind almost as much trouble as it must have once given the turtle." (Pope, '39). In fact, turtles must have had another mechanism for breathing that was independent of the primitive costal ventilation mechanism before they fused the ribs into the shell.

We hypothesize that the respiratory functions of the abdominal muscles of turtles evolved, as many accessory ventilation mechanisms appear to have, in response to the primitive mechanical conflict between respiration and locomotion hypothesized to have been present in early amniotes (Carrier, '87, '91). Duplication of ventilatory function before evolving the shell would then have set the stage for the ribs to abandon their role in lung ventilation.

This hypothesis is supported by the conclusion that the abdominal muscles seem to play a key role in the remarkable and unexpected finding that there is no measurable phase coupling between the respiratory and locomotor cycles in two emydid turtles. The actions of the abdominal muscles appear to dampen any effects of limb movements, effectively decoupling the locomotor and breathing cycles. The complexity of interactions between ventilation and locomotion in turtles seems to grow with each study, as the three most-carefully studied species have revealed different modifications of breathing during locomotion.

#### ACKNOWLEDGMENT

We thank Jim O'Reilly for lending us the turtles for this study. Scott Currie generously provided us with figures of unpublished work. Mark Mandica prepared the turtle drawings used in Figure 2. Emily Jerome edited the manuscript. This material is based on work supported by the US National Science Foundation under grants 9875245 and 0316174 to E. L. B.

#### LITERATURE CITED

- Agassiz LD. 1857. Contributions to the natural history of the United States, Vol. 1. Boston: Little Brown.
- Batschelet E. 1981. Circular statistics in biology. London: Academic Press.
- Brainerd EL, Owerkowicz T. 2006. Functional morphology and evolution of aspiration breathing in tetrapods. *Respir Physiol Neurobiol* 154:73-78.
- Carrier DR. 1987. The evolution of locomotor stamina in tetrapods: circumventing a mechanical constraint. *Paleobiology* 13:326-341.
- Carrier DR. 1991. Conflict in the hypaxial musculo-skeletal system: documenting an evolutionary constraint. *Amer Zool* 31:644-654.
- Crawford EC, Gatz RN, Magnussen H, Perry SF, Piiper J. 1976. Lung volumes, pulmonary blood flow and carbon monoxide diffusing capacity of turtles. *J Comp Physiol* 107:169-178.
- Currie SN. 2001. Turtle "respiratory muscles" switch from a synchronous to an alternating pattern of activity during the changeover from breathing to swimming. *Soc Neurosci Abstr* 27:830.12.
- Currie SN. 2003. Bilateral activity patterns of pelvic respiratory muscles during breathing and swimming in turtles. *J FASEB*. 17 Abstract #793.1 (Suppl A1210).
- Druzisky KA, Brainerd EL. 2001. Buccal oscillation and lung ventilation in a semi-aquatic turtle, *Platysternon megacephalum*. *Zoology* 104:143-152.
- Earhart GM, Stein PSG. 2000. Scratch-swim hybrids in the spinal turtle: blending of rostral scratch and forward swim. *J Neurophysiol* 83:156-165.
- Farmer CG, Hicks JW. 2000. Circulatory impairment induced by exercise in the lizard *Iguana iguana*. *J Exp Biol* 203:2691-2697.
- Gans C, Hughes GM. 1967. The mechanism of lung ventilation in the tortoise *Testudo graeca* Linne. *J Exp Biol* 47:1-20.
- Gaunt AS, Gans C. 1969. Mechanics of respiration in the snapping turtle, *Chelydra serpentina* (Linné). *J Morphol* 128:195-228.
- George JC, Shaw RV. 1959. The structural basis of the evolution of the respiratory mechanism in Chelonia. *J Anim Morphol Physiol* 1:1-9.
- Gilbert SF, Cebra-Thomas JA, Burke AC. 2008. How the turtle got its shell. In: Wyneken J, Godfrey MH, Bels V, editors. *Biology of turtles*. Boca Raton, FL: CRC Press. p 1-16.
- Jackson DC. 1971. The effect of temperature on ventilation in the turtle, *Pseudemys scripta elegans*. *Respir Physiol* 12:131-140.
- Jackson DC, Prange HD. 1979. Ventilation and gas exchange during rest and exercise in adult green sea turtles. *J Comp Physiol* 134:315-319.
- Kidd C, Brainerd EL. 2000. Abdominal pressure during high speed locomotion in the Texas spiny lizard, *Sceloporus olivaceus*. *Amer Zool* 40:1085A.
- Landberg T, Mailhot JD, Brainerd EL. 2003. Lung ventilation during treadmill locomotion in a terrestrial turtle, *Terrapene carolina*. *J Exp Biol* 206:3391-3404.
- McCutcheon FH. 1943. The respiratory mechanism in turtles. *Physiol Zool* 3:255-269.
- Mitchell SW, Morehouse GR. 1863. Researches upon the anatomy and physiology of respiration in the Chelonia. *Smithsonian Contrib Knowl* 159:1-42.
- Munns SL, Hartzler LK, Bennett AF, Hicks JW. 2005. Terrestrial locomotion does not constrain venous return in the American alligator, *Alligator mississippiensis*. *J Exp Biol* 208:3331-3339.

- Pope CH. 1939. Turtles of the United States and Canada. New York: Alfred A. Knopf.
- Prange HD, Jackson DC. 1976. Ventilation, gas exchange and metabolic scaling of a sea turtle. *Respir Physiol* 27: 369-377.
- Shaw RV. 1962. A comparative study of the respiratory muscles in Chelonia. *Brevoria* 161:1-16.
- Stein PS, Camp AW, Robertson GA, Mortin LI. 1986. Blends of rostral and caudal scratch reflex motor patterns elicited by simultaneous stimulation of two sites in the spinal turtle. *J Neurosci* 6:2259-2266.
- Tresch MC, Saltiel P, d'Avella A, Bizzi E. 2002. Coordination and localization in spinal motor systems. *Brain Res Rev* 40:66-79.
- Vitalis TZ, Milsom WK. 1986a. Pulmonary mechanics and the work of breathing in the semi-aquatic turtle, *Pseudemys scripta*. *J Exp Biol* 125:137-155.
- Vitalis TZ, Milsom WK. 1986b. Mechanical analysis of spontaneous breathing in the semi-aquatic turtle, *Pseudemys scripta*. *J Exp Biol* 125:157-171.
- Zar JH. 1996. Biostatistical analysis. Upper Saddle River, NJ: Prentice-Hall.