

PELVIC ASPIRATION IN THE AMERICAN ALLIGATOR (*ALLIGATOR MISSISSIPPIENSIS*)

COLLEEN G. FARMER^{1,*} AND DAVID R. CARRIER²

¹Department of Ecology and Evolutionary Biology, 321 Steinhaus Hall, University of California, Irvine, CA 92697, USA and ²Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112-0840, USA

*e-mail: cfarmer@uci.edu

Accepted 22 March; published on WWW 10 May 2000

Summary

The pelvis of crocodylians is highly derived in that the pubic bones are isolated from the acetabulum and are attached to the ischia *via* moveable joints. We examined the possible role of this unusual morphology in lung ventilation by measuring ventilation, abdominal pressure and the electrical activity of several abdominal and pelvic muscles in the American alligator (*Alligator mississippiensis*). We found that the activity of two pelvic muscles, the ischiopubis and ischiotruncus muscles, was correlated with inspiration; these muscles rotate the pubes ventrally and thereby increase abdominal volume. During expiration,

contraction of the rectus abdominis and transversus abdominis rotates the pubes dorsally. We suggest that this mechanism facilitates diaphragmatic breathing by creating space for caudal displacement of the viscera during inspiration. Because birds also use a dorso-ventral movement of the pelvis to effect ventilation, some form of pelvic aspiration may be plesiomorphic for archosaurs.

Key words: lung ventilation, alligator, *Alligator mississippiensis*, ventilatory mechanics, evolution, electromyography.

Introduction

Crocodylians have a unique mechanism of lung ventilation that employs a hepatic piston, driven by a diaphragmatic muscle (Boelaert, 1942; Gans and Clark, 1976; Naifeh et al., 1970a). The diaphragmatic muscle originates on the pelvis and caudoventral body wall and extends forward to envelop the lateral and ventral portions of the liver. When the muscle shortens, it pulls the liver caudally and thereby increases the volume of the pleural cavities. Subsequent contraction of the transversus abdominis muscle reduces the volume of the abdominal cavity, forcing the liver cranially, and thereby produces expiration. The ribs and intercostal muscles also contribute to changes in the volume of the pleural cavities, but lung ventilation can be produced almost exclusively by movements of the hepatic piston (Gans and Clark, 1976). Diaphragmatic breathing of crocodylians is analogous to diaphragmatic breathing in mammals because both systems increase the volume of the pleural cavities by displacing the viscera caudally. It has been suggested that diaphragmatic breathing in crocodylians is associated with an amphibious lifestyle (Gans and Clark, 1976) and with an ability to breathe during terrestrial locomotion (Carrier, 1987a).

Crocodylians may possess another means of ventilating their lungs that has not been investigated previously. Dissections of *Caiman crocodilus* and *Alligator mississippiensis* have led us to suspect that the pelvic girdle of crocodylians is not simply a passive anchor for the diaphragmatic muscle, but is kinetic

in a manner that facilitates the pumping of air into and out of the lungs. The pubic bones of crocodylians are unusual because they do not form part of the acetabulum and they are attached to the ischia *via* moveable joints (Romer, 1956). From these joints, the pubes extend cranially and ventrally to form expanded horizontal plates that lie along the ventral body wall of the caudal abdominal cavity. Manual manipulation suggests that ventral rotation of the pubic bones would increase abdominal volume, whereas dorsal rotation would have the opposite effect. Furthermore, when a young alligator is held in the palm of one's hand, a ventral expansion of the caudal abdomen is felt during inspiration.

There are four muscles whose action could serve to rotate the pubic bones ventrally. The pubo-ischio-femoralis externus muscle originates on the proximal femur and extends cranially to attach to the dorsal and ventral surfaces of the horizontally expanded pubic bones (Romer, 1923). Because this muscle lies slightly ventral to the iliac–pubic joint, contraction will tend to rotate the pubes ventrally. Three other muscles lie along the ventral margin of the pelvis and could expand the abdominal cavity through ventral rotation of the pubes: the truncocaudalis (Maurer, 1896), ischiopubis and ischiotruncus. These muscles originate on the ventral surface of the proximal tail muscles or on the ventral surface of the ischium and extend anteriorly to attach to the cranial margin of the pubes and the most caudal gastralia. These muscles are relatively small in cross section,

but they have large moment arms at the ischio-pubic joint. Contraction of any of these muscles could rotate the pubes ventrally, which would increase the volume of the body cavity.

To test whether the pelvic musculo-skeletal system of crocodylians contributes to lung ventilation, we recorded ventilation, abdominal pressure and the activity of several muscles during rest and terrestrial locomotion in six specimens of *Alligator mississippiensis*.

Materials and methods

Animals

American alligators (*Alligator mississippiensis*) were kept in aquaria with basking platforms, a thermal gradient and a full-spectrum light source. They experienced a photoperiod of 14h:10h light:dark. They were fed a diet of goldfish, smelts, mice, rats and eggs. The mass of the animals ranged from 1.18 to 2.68 kg. Six animals were trained to walk at a speed of 0.31 m s^{-1} on a treadmill over the course of several months.

An anatomical description of the pelvic musculature was obtained by dissecting five alligators that ranged in mass from 1.18 to 2.2 kg.

Lung ventilation

To measure ventilation, a mask was constructed from the tip of a 30 ml plastic syringe. Two ports were drilled into the syringe, and flexible Hytrel tubing (Hans Rudolph, Inc., Kansas City, MO, USA) with an inner diameter of 9 mm was glued to the ports. The mask was sealed over the nares with quick-setting epoxy adhesive. The mouth of the animal was sealed with duct tape. Fresh air was pulled through the mask at a rate of 5.41 min^{-1} by a vacuum. This was the biased flow rate determined to eliminate rebreathing by the alligators during their most vigorous breathing. A pneumotachometer (8311, Hans Rudolph, Inc., Kansas City, MO, USA) was placed in the line upstream from the mask. The pneumotachometer was calibrated by injecting known volumes of air into the sealed mask before it was attached to the animal.

Ventilation was measured during rest, during walking on a treadmill and during recovery from walking exercise. We also induced vigorous ventilation in resting alligators by increasing the concentration of CO_2 in the inspired air to 10%. Controls, in which the subjects did not wear the mask, demonstrated that the presence of the mask did not influence the pattern of muscle recruitment; the timing, duration and intensity of muscle activity appear to be the same.

Stride

A mercury strain gauge, wired to one arm of a Wheatstone bridge, was mounted to the back of each animal using Velcro. Bending of the trunk during walking stretched the gauge, altered the resistance of the mercury column and thus provided an indication of the locomotor cycle.

Abdominal pressure

Abdominal pressure was measured using a Millar Mikro-Tip

pressure transducer system (Millar Instruments, Inc., Houston, TX, USA). The pressure transducers were calibrated before insertion into the abdomen. Calibration was also checked after the experiment, upon removal of the transducer. The tips of the transducers were positioned dorsal to both the diaphragmatic muscle and the pubic bones, and they were secured by suture to the ventral body wall.

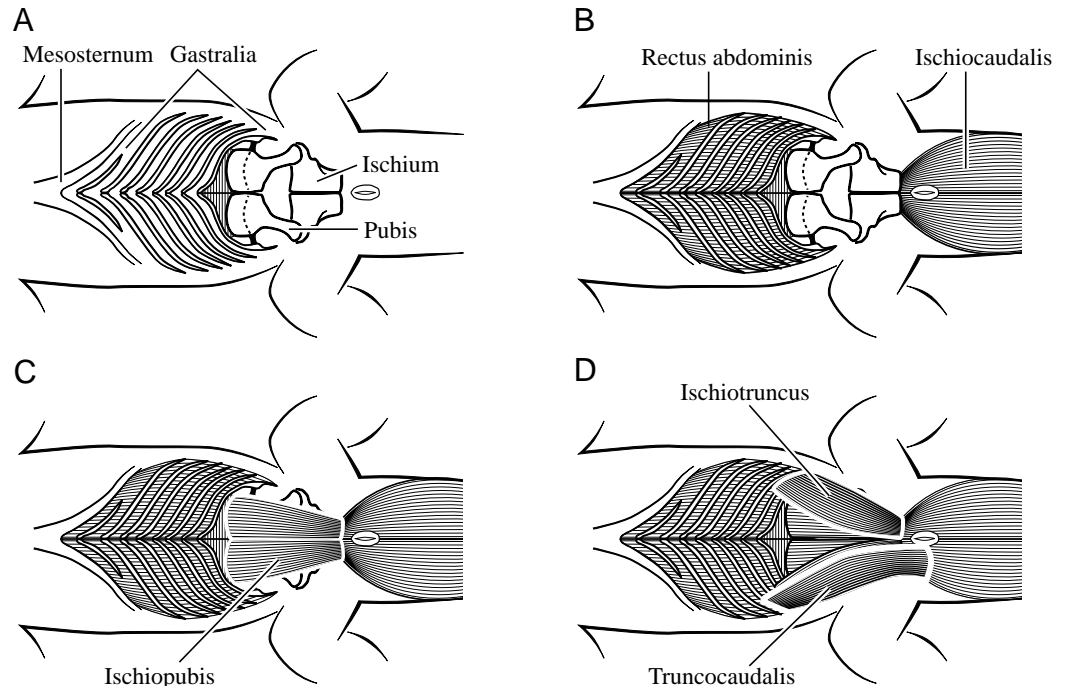
Electromyography

We recorded the electrical activity of eight muscles: transversus abdominis ($N=4$ animals), rectus abdominis ($N=3$), diaphragmaticus ($N=4$), truncocaudalis ($N=3$), ischiopubis ($N=5$), ischiotruncus ($N=3$) and the dorsal and ventral heads of the pubo-ischio-femoralis externus ($N=1$). We used sew-through electrodes constructed and implanted as described previously (Betts et al., 1976; Carrier, 1996). Two electrodes were placed in each muscle to ensure the quality of the signals. Lead wires from the electrodes and ground wires were tunneled subcutaneously to a dorsal exit just caudal to the hindlimb. At the exit, the wires passed through a silastic tube sutured to the skin. The tube was then filled with silicone sealant, which provided a mechanical anchor for the wires and a barrier to pathogens. The alligators were allowed 2 days to recover from surgery before recordings were made. Electromyographic signals were passed through shielded, lightweight cables (Cooner Wire Inc., Chatsworth, CA, USA) to a.c. amplifiers (Grass P5, Quincy, MA, USA). The electromyographic signals were filtered above 1000 Hz and below 30 Hz, and they were amplified 5000–10 000 times.

To determine an appropriate sampling rate for data acquisition, we sampled signals from the ischiopubis and rectus abdominis muscles at 6000 Hz and used Fast Fourier Transformation to find the dominant frequencies of the signals. During locomotion, the mean and standard deviation of the dominant frequency of the ventilatory signals from the ischiopubis and rectus abdominis muscles were 186 ± 28 and 191 ± 20 Hz ($N=5$) respectively. Given these relatively low frequencies, we chose a sampling rate of 1750 Hz. This allowed us to sample 10 channels simultaneously for extended periods and provided an effective sampling of more than eight samples per spike for the dominant frequency of the signals produced by the muscles.

Three of the muscles were adjacent to other muscles that were potential sources of electrical cross-talk. To guard against cross-talk, the electrodes in these muscles were insulated from the adjacent muscle with thin sheets of silastic rubber. The recording site for the transversus abdominis muscle was at the mid-abdomen. To eliminate possible cross-talk from the diaphragmatic muscle, we sutured a large sheet ($1.5 \text{ cm} \times 2 \text{ cm}$) of silastic between the transversus abdominis and the diaphragmaticus. The electrode sites for the diaphragmaticus and rectus abdominis muscles were also mid-abdomen, near the mid-line of the body. An effort was made to place the rectus abdominis electrodes directly ventral to the placement of the diaphragmaticus electrodes. This allowed the electrodes in these two muscles to be insulated against cross-

Fig. 1. Ventral musculature of the pelvis and abdomen of the American alligator (*Alligator mississippiensis*). (A) Illustration of the ventral aspect of the pelvis and gastralia. The cranial halves of the pubic plates are composed of cartilage in juvenile alligators. The pubic plates are attached to the last gastralium through a collagenous sheet (anteriorly) and through a lateral ligament. (B) Rectus abdominis and ischio-caudalis muscles. (C) Ischiopubis muscle. (D) The bottom half of the figure illustrates the relationship between the truncocaudalis muscle and the ischiotruncus muscle above it. In the top half of the figure, the truncocaudalis muscle has been removed to show the ischiotruncus muscle.



talk with a silastic sheet (2 cm×2 cm) sutured between the muscles.

Data collection and analysis

The analog signals from the pneumotachometer, the electromyogram (EMG) amplifiers, the mercury strain gauge and the pressure transducer were converted to digital format using a BioPac System (Goleta, CA) and sampled at 1750 Hz with AcqKnowledge software (BioPac, Goleta, CA, USA).

To test the correlation between the activity of a particular muscle and the production of ventilatory air-flow, we rectified and integrated the EMG signals on a breath-by-breath basis for the rectus abdominis, ischiopubis, ischiotruncus and diaphragmaticus muscles. We then used least-squares power regression to determine the level of correlation between the integrated area for each of these muscles and tidal volume. This analysis assumes that the integrated area of the EMG signals provides a measure of the level of recruitment of the muscle (Loeb and Gans, 1986). We wish to emphasize that the primary intent of this analysis was to test for the presence or absence of a correlation between the amount of muscle activity and the size of the breaths. Given the differences between electrodes, sites of implantation and individual recruitment patterns, the equations generated from this analysis should not be compared among animals or muscles.

Results

Anatomy

Pelvis

The pubes of crocodylians are totally excluded from the borders of the acetabulum by a cranial expansion of the

ischium and by a mass of fibrocartilage that forms the anterior margin of the acetabulum (Romer, 1956). From these joints, the pubes extend cranially and ventrally (Fig. 1). Distally, they expand to become horizontal plates that are partially cartilaginous in young specimens. The two plates are joined at the midline through a loose ligamentous attachment. The plates are also attached to the caudal-most gastralium, anteriorly by a fibrous sheet and laterally by a ligamentous band (Fig. 1). Together, the pubes form the posterior and ventral margins of the body cavity.

With the exceptions of the ischiopubis and ischiotruncus muscles, the pelvic muscles of *Alligator mississippiensis* have been described previously (Romer, 1923). Here, we provide a brief description of the muscles addressed in the present study.

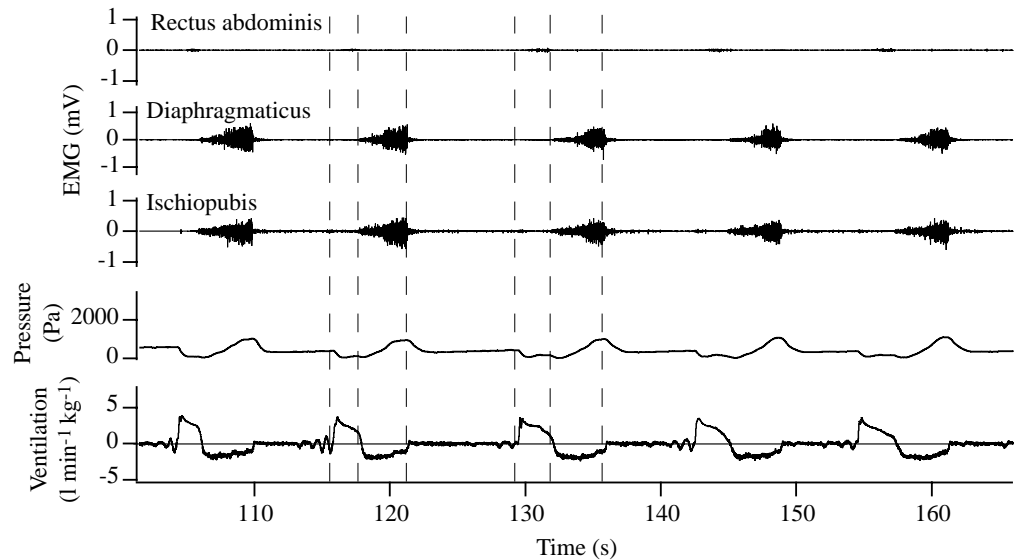
Diaphragmaticus muscle

This muscle is described by Boelaert (1942), Gans and Clark (1976) and Naifeh et al. (1970a). It is a thin sheet of muscle that envelops the abdominal cavity and extends from the pelvis to the liver. The lateral portions of the muscle originate on the dorso-lateral portion of the ischia. The ventral portions of the muscle originate on the last (i.e. most caudal) gastralium. The diaphragmaticus inserts on a collagenous sheath that envelops the liver. The ventral origin of this muscle in *Alligator mississippiensis* differs from that described by Gans and Clark (1976) for *Caiman crocodilus*, in which the muscle attaches to the distal portion of the pubis rather than to the last gastralium.

Truncocaudalis muscle

This muscle is the posterior portion of the rectus abdominis (Maurer, 1896; Romer, 1923). Caudally, it arises from the ventral surface of the ischio-caudalis muscle, just lateral to the

Fig. 2. Sample recording of the activity of the rectus abdominis, diaphragmaticus and ischiopubis muscles (EMG) plotted relative to changes in abdominal pressure and ventilatory air-flow from an American alligator. Data are from alligator 4 (body mass 1.18 kg) during a recovery period immediately after exercise. In the ventilation recording, expiration occurs when the trace is above zero and inspiration occurs when the trace is below zero. Dashed vertical lines mark the beginning and end of the expiratory and inspiratory phases of ventilation.



cloaca (Fig. 1). It extends cranially, ventral to the pelvic girdle, and inserts on the lateral margins of the last four gastralia and the fascia of the rectus abdominis and external oblique muscles.

Ischiotruncus muscle

This muscle originates on the ventral, posteriolateral margin of the ischium (Fig. 1). It forms a thin sheet that passes ventral to the pubis and attaches to the last two gastralia. Its major site of insertion is on the broad bend of the last gastralia. The lateral portion of the muscle is dorsal to the truncocaudalis muscle.

Ischiopubis muscle

Immediately dorsal and medial to the ischiotruncus muscle is a second thin layer of muscle, the ischiopubis muscle (Fig. 1). This layer also originates on the ventral, posteriolateral margin of the ischium. It extends forward to attach to the cranial margin of the pubis.

Pubo-ischio-femoralis externus

This complex is described in detail by Romer (1923) and is mentioned only briefly here. One portion arises from the dorsal surface of the pubis, a second portion arises from the ventral surface of the pubis, and the third segment arises from the ischium (not shown in Fig. 1). The three heads unite in a tendinous insertion on the posterior ventral margin of the femur adjacent to the head.

Rectus abdominis

This muscle is clearly described by Romer (1923). It extends from the posterior margin of the sternum to the last gastralia (Fig. 1). Along its length, gastralia interrupt its ventral portion, whereas gastralia are not apparent on its dorsal surface. None of the muscle fibers attaches directly to the pubis. Instead, connection to the pelvis is made medially through the ligamentous sheet that attaches the last gastralia to the pubes

and laterally through a strong ligament between the last gastralia and the lateral edge of the pubis.

Transversus abdominis

In the abdomen, the fibers of this muscle arise from the transverse fascia and run ventrally to the level of the lateral edges of the gastralia. The muscle continues to extend ventrally as a tendinous sheet to the ventral midline. In the thorax, the transversus abdominis arises from the proximal portion of the dorsal ribs and extends ventrally to the distal portion of the abdominal ribs. Hence, it crosses the joints between the dorsal ribs and the abdominal ribs and the joints in the abdominal ribs.

Muscle activity

Of the eight muscles from which we recorded, activity of the diaphragmaticus (Figs 2–4), ischiopubis (Figs 2–4) and ischiotruncus muscles was correlated with inspiration. The diaphragmaticus and ischiopubis muscles exhibited strong, phasic activity during inspiration in every breath we recorded. This was true during rest, walking, recovery from exercise and during elevated ventilation induced by increased levels of CO₂ in the inspired air. In contrast, in two of the three alligators (alligators 5 and 6), the ischiotruncus muscle appeared to be an accessory muscle of inspiration; exhibiting significant activity only in response to elevated CO₂ levels in the inspired air. In the third subject (alligator 2), however, inspiration was always correlated with activity of the ischiotruncus muscle. Activity of the rectus abdominis (Figs 2, 3) and transversus abdominis (Fig. 4) muscles was associated with expiration in every animal in which we monitored these muscles. Patterns of muscle activity during recovery from exercise are summarized in Fig. 5 for those muscles that appear to be associated with ventilation.

The integrated area of the electromyographic signals was strongly correlated with tidal volume in the rectus abdominis muscles, ischiopubis muscles and diaphragmaticus muscles (Table 1). The integrated EMG activity of the ischiotruncus

Fig. 3. Sample recording of the activity of the rectus abdominis, diaphragmaticus and ischiopubis muscles (EMG) plotted relative to changes in abdominal pressure, ventilatory air-flow and the timing of the locomotor cycle from an American alligator walking at 0.31 m s^{-1} . Data are from the same trial of alligator 4 as shown in Fig. 2. Note the differences in intensity of the electromyographic signals during walking compared with recovery. In the ventilation recording, expiration occurs when the trace is above zero and inspiration occurs when the trace is below zero. The bottom trace is a recording of the lateral bending of the trunk; each cycle of the trace represents a complete locomotor cycle. Dashed vertical lines mark the beginning and end of the expiratory and inspiratory phases of ventilation.

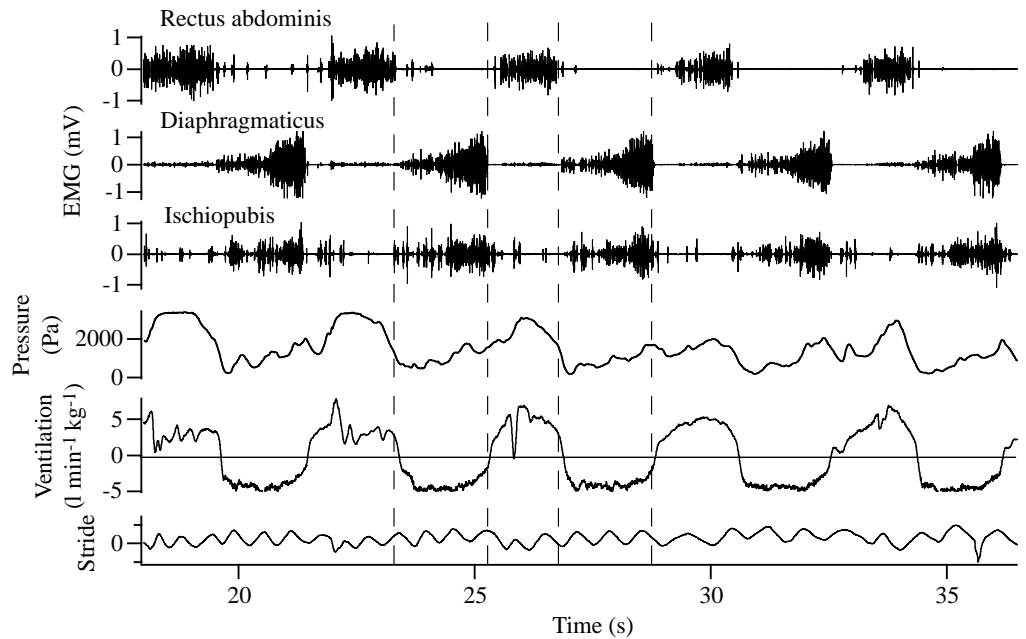
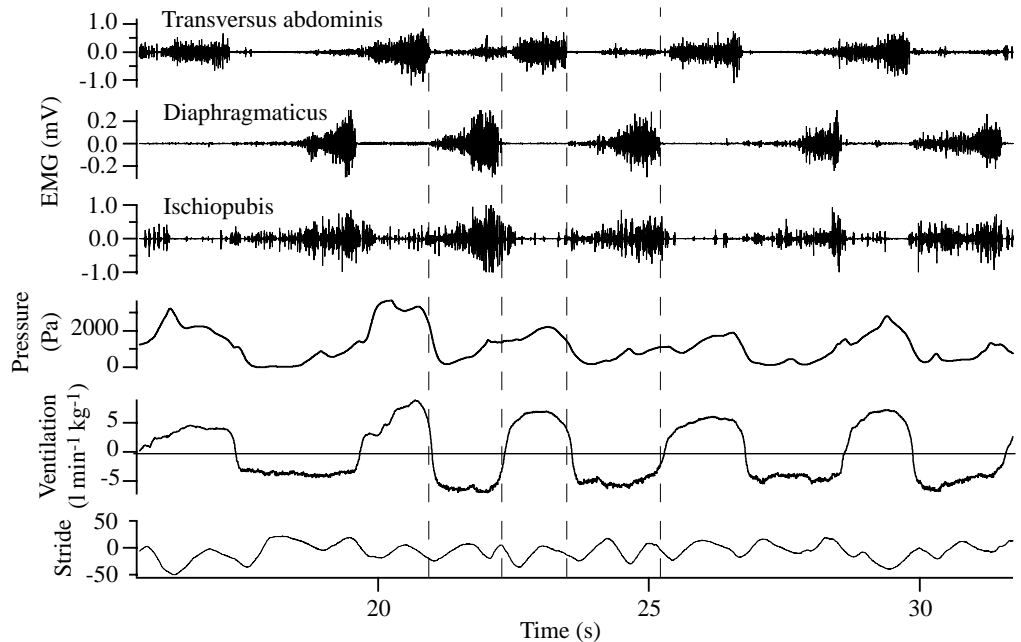


Fig. 4. Sample recording of the activity of the transversus abdominis, diaphragmaticus and ischiopubis muscles (EMG) plotted relative to changes in abdominal pressure, ventilatory air-flow and the timing of the locomotor cycle from an American alligator walking at 0.31 m s^{-1} . Data are from alligator 2 (body mass 1.26 kg). In the ventilation recording, expiration occurs when the trace is above zero and inspiration occurs when the trace is below zero. The bottom trace is a recording of the lateral bending of the trunk; each cycle of the trace represents a complete locomotor cycle. Dashed vertical lines mark the beginning and end of the expiratory and inspiratory phases of ventilation.



muscle was strongly correlated with tidal volume in alligator 2, weakly correlated in alligator 6 and not correlated in alligator 5 (Table 1). The poor correlation between the activity of this muscle and tidal volume was a function of the fact that, in two of the subjects, it exhibited significant activity only during vigorous breathing induced by elevated CO_2 levels in the inspired air. It warrants mention that, because of subtle differences in electrode design, electrode location within a muscle and inter-individual variation in motor recruitment, the regression equations presented in Table 1 should not be

compared among individuals or among muscles. The relevant observation is the presence or absence of a positive correlation between tidal volume and integrated muscle activity in an individual muscle.

Three muscles that have a capacity to cause ventral rotation of the pubic bones appear not to be associated with inspiration. The ventral and dorsal heads of the pubo-ischio-femoralis externus muscle showed clear phasic activity associated with the locomotor cycle, but displayed no activity correlated with ventilation. Consequently, we monitored the pubo-ischio-

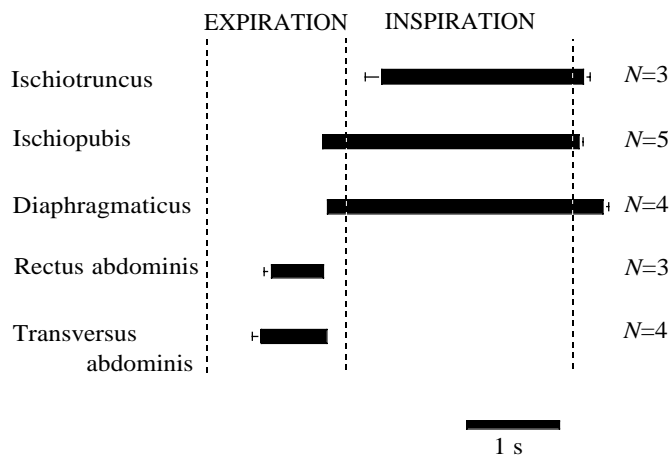


Fig. 5. Summary of the timing of the electromyographic activity of five ventilatory muscles of the American alligator. Rectangles and error bars represent means and 1 S.E.M. of the onset and offset times of muscle activity plotted relative to the timing (vertical lines) of expiratory and inspiratory air-flow during recovery from exercise. Note that the data for the ischiotruncus muscle include recordings from two individuals breathing elevated levels of CO_2 . The number of individuals from which data were analyzed is presented on the right side of the graph for each muscle. Four breaths were analyzed for each individual.

femoralis muscle in a single animal only. The truncocaudalis muscle exhibited low-level phasic activity associated with inspiration in resting alligators and during the vigorous breathing induced by elevated levels of CO_2 in the inspired air, but its relationship to ventilation could not be determined during locomotion because the intensity of activity associated with the stride was of a much greater level.

The expiratory activity from the rectus abdominis (Figs 2, 3) and transversus abdominis muscles was of very low level during rest and during recovery from exercise. Although these muscles always displayed activity associated with expiration, activity was often discernible only late in the expiratory phase. In contrast, both muscles displayed more intense activity when the animals exhaled during exercise (Figs 3, 4). During walking, both muscles became active at the beginning of expiration and remained active throughout the period of expiratory air-flow. The relatively low level of activity of the rectus abdominis muscle during resting ventilation is apparent in the low slopes of the power regressions for this muscle (Table 1). Slope values less than 1.0 indicate that relatively less muscle activity was recorded for small breaths than for large breaths.

The rectus abdominis muscle did not exhibit any activity that could be associated with the locomotor cycle (Fig. 3). The transversus abdominis muscle, however, occasionally exhibited low-level activity correlated with the stride. This activity in the transversus abdominis muscle was of a much lower level than that associated with ventilation (Fig. 4). Thus, both the rectus abdominis and transversus abdominis muscles appear to be primarily ventilatory in their function.

The activity of the diaphragmaticus and ischiopubis muscles

Table 1. Least-squares regressions of tidal volume against the integrated area of the electromyographic activity of four respiratory muscles

Muscle	Alligator					
	number	<i>a</i>	<i>b</i>	r^2	<i>N</i>	<i>P</i> value
Rectus abdominis	2	506.8	0.51	0.77	18	<0.0001
	3	379.0	0.48	0.43	17	0.0043
	4	409.3	0.86	0.81	28	<0.0001
Ischiopubis	1	5758.6	1.65	0.63	23	<0.0001
	3	257.5	0.57	0.88	17	<0.0001
	4	863.3	1.08	0.88	28	<0.0001
	5	648.6	0.73	0.50	30	<0.0001
	6	4083.5	1.15	0.73	26	<0.0001
	Diaphragmaticus	1	506.5	0.83	0.94	23
Diaphragmaticus	2	507.4	0.83	0.76	18	<0.0001
	3	151.4	0.40	0.83	17	<0.0001
	4	409.3	0.86	0.86	28	<0.0001
	Ischiotruncus	2	582.5	0.96	0.91	18
Ischiotruncus	5	32.5	-0.14	0.01	24	0.6320
	6	243.2	0.29	0.38	28	0.0005

Equations are of the form $y = ax^b$, where x is the integrated area of muscle activity (mV s) and y is expiratory tidal volume (ml kg^{-1}).

was characterized by a gradual increase in intensity throughout the inspiratory period (Figs 2–4). Both muscles became active at the start or slightly before the beginning of inspiration. The increase in intensity of the activity during inspiration was most pronounced during resting ventilation, but was also apparent during exercise. The diaphragmaticus muscle exhibited no activity that could be associated with locomotor movements. In contrast, the ischiopubis and ischiotruncus muscles often exhibited faint and intermittent activity correlated with the locomotor cycle, turning on briefly coincident with the footfall of the ipsilateral hindlimb.

Ventilation and abdominal pressure

The pattern of lung ventilation during walking differed from that observed before exercise (i.e. rest) and during recovery from exercise. A detailed description of ventilatory air-flow before, during and after exercise is given elsewhere (Farmer and Carrier, 2000a,b); only a brief summary follows. Before and after exercise, individual breaths were comparatively long (approximately 6–9 s) and were separated by periods of apnea of variable duration (Fig. 2). During walking, respiratory flow rates were greater and the total duration of inspiration and expiration was reduced to approximately 3 s (Figs 3, 4). In addition, breaths often occurred in quick succession, without periods of apnea. Both tidal volume and respiratory frequency were significantly elevated during exercise. Consequently, ventilation during exercise was much more forceful than during quiet breathing or during recovery from exercise (Farmer and Carrier, 2000a,b).

Before and after exercise, abdominal pressures mirrored the changes in ventilation. Shortly after the initiation of expiration, abdominal pressure decreased. Abdominal pressure remained

depressed throughout the period of expiration and often began to drop further shortly before the end of expiration and the beginning of inspiration. The lowest pressures were associated with the beginning of the inspiratory phase. Inspiration was characterized either by a plateau followed by a sharp rise or by a gradual rise in abdominal pressure throughout the inspiratory phase. Peak pressure always occurred at the end of inspiration. Pressure then generally dropped slightly to a steady level during the beginning of the apneic period. Abdominal pressures during periods of apnea were always elevated above ambient (496 ± 96 Pa, mean \pm S.E.M., $N=4$).

During walking, the absolute values of abdominal pressure were highly variable. Nevertheless, a clear pattern relative to the phase of ventilation could be discerned. During locomotion, abdominal pressure was minimal at the beginning of inspiration and rose as inspiration progressed. In contrast to the pre-exercise pattern, pressure continued to rise during the beginning of expiration, and peak abdominal pressures were always observed during expiration.

Discussion

How crocodilians breathe

Crocodylians devote a surprising amount of their musculo-skeletal anatomy to the function of lung ventilation, and the mechanism of ventilation appears to be more complex than has previously been realized. The breath cycle begins with expiration. Associated with expiratory air-flow is activity in the intercostal musculature (Gans and Clark, 1976), the transversus abdominis muscle (Gans and Clark, 1976; Naifeh et al., 1970a; this study) and the rectus abdominis (this study) muscles. Although the rectus abdominis and transversus abdominis muscles are always active during expiration in resting animals, the relatively low level of activity of these muscles and the drop in abdominal pressure at the beginning of expiration suggest that expiration is largely passive when alligators are resting. During exercise, however, expiration is clearly an active process; the intensity of expiratory muscle activity increases many times above that observed at rest, and abdominal pressure rises to peak levels early in the expiratory period.

Inspiration in crocodylians is characterized by activity in the intercostal musculature (Gans and Clark, 1976), the diaphragmaticus muscle (Gans and Clark, 1976; Naifeh et al., 1970a; this study), the ischiopubis muscle (this study) and the ischiotruncus muscle as an accessory inspiratory muscle (this study). The intensity of activity in these muscles during inspiration indicates that inspiration is an active process during both rest and exercise.

From these observations, a general description can be drawn of the mechanism of ventilation in crocodylians (Fig. 6). Our recordings are consistent with previous work that shows that alligators are entirely aspiration breathers. They do not inflate their lungs through buccal pumping (Gans and Clark, 1976; Huggins et al., 1968; Naifeh et al., 1970a,b, 1971). Expiration is produced (i) by activity of the intercostal muscles, which pull

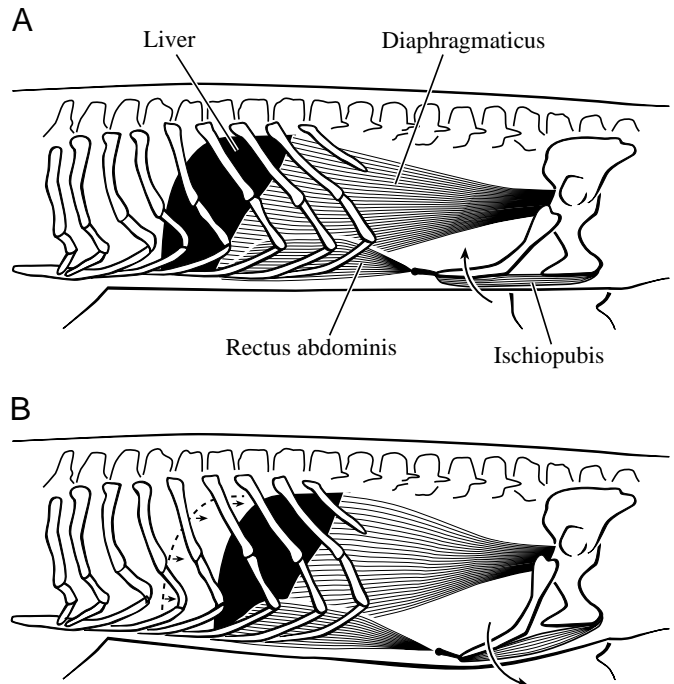


Fig. 6. Illustration of the mechanism of ventilation in the American alligator. (A) Expiration is produced by caudal rotation of the ribs and constriction of the abdominal cavity, which produces a cranial translation of the viscera. Constriction of the abdomen results from activity of the transversus abdominis muscle and the rectus abdominis muscle, which rotate the pubes dorsally. (B) Inspiration is produced by cranial rotation of the ribs and caudal translation of the viscera. The viscera are pulled posteriorly by contraction of the diaphragmaticus muscle. The ischiopubis muscle increases the volume of the posterior abdomen by rotating the pubes ventrally.

the ribs caudally and medially to decrease the volume of the thorax, as is the case in most amniotes; (ii) by activity of the transversus abdominis muscle, and possibly other body wall muscles, which decreases the volume of the abdomen and shifts the liver cranially, reducing the volume of the thoracic cavity; and (iii) by activity of the rectus abdominis muscle, and possibly the transversus abdominis muscle, which rotates the pubic bones in the cranial-dorsal direction, decreasing the volume of the abdominal cavity. This also aids in shifting the liver cranially. Inspiration is produced (i) by cranial rotation of the ribs caused by activity of the intercostal muscles; (ii) by caudal translation of the abdominal viscera caused by contraction of the diaphragmaticus muscle; and (iii) by posterior abdominal expansion, caused by contraction of the ischiopubis and ischiotruncus muscles. We believe that these two muscles function to expand the posterior abdomen to make space for the caudal displacement of the viscera as a result of the action of the diaphragmaticus muscle. Although we have no direct recordings of movements of the pubic bones, videofluoroscopy recordings made recently by Dr Elizabeth Brainerd and Tomasz Owerkowicz confirm that the pubic bones of young American alligators rotate ventrally during inspiration (E. Brainerd and T. Owerkowicz, personal communication).

Abdominal pressure

During rest and prior to exercise, when breathing is relatively calm, expiration appears to be primarily a passive process. Levels of muscle activity in the rectus abdominis and transversus abdominis muscles were much lower during resting expirations than during exercise expirations. This, combined with a drop in abdominal pressure to approximately ambient pressure at the start of expiration, suggests that expiration is primarily passive when the animals are resting. In contrast, during exercise, both the rectus abdominis and transversus abdominis exhibit high levels of activity during expiration. Associated with this expiratory muscle activity is an increase in abdominal pressure. During exercise, the highest abdominal pressures occurred during expiration.

In contrast to the pattern during expiration, abdominal pressure dropped during the beginning of inspiration. Because the diaphragmaticus muscle envelops the viscera ventrally and laterally, one might expect that contraction of the diaphragmaticus during inspiration would result in an immediate increase in abdominal pressure as the muscle squeezes the viscera and translates the liver posteriorly. However, this was not observed. Instead, we found a decrease in abdominal pressure during the beginning of the inspiratory phase. This can only be explained by an expansion of the abdominal cavity. We suspect that this expansion is caused by the ventral rotation of the pubes. Abdominal pressures rise during the later part of inspiration. This is probably due to a decrease in the rate of expansion of the abdominal cavity coupled with the posterior movement of the viscera and liver.

In contrast to lizards, in which the abdominal viscera are sucked towards the thorax during inspiration (i.e. paradoxical breathing), it has long been appreciated that both the abdomen and thorax in crocodilians and mammals expand laterally during inspiration (Boelaert, 1942). In mammals, the relaxation of abdominal muscles concurrent with contraction of the diaphragm causes the abdomen to flare outwards and abdominal pressure to drop (Ainsworth et al., 1989; Youmans et al., 1963). The lateral abdominal movement in crocodilians has also been attributed to the diaphragmaticus muscle pulling the liver caudally which, in turn, causes the abdominal viscera to be displaced laterally (Boelaert, 1942). However, the action of the diaphragmaticus muscle of crocodilians differs from that of the mammalian diaphragm because the crocodilian diaphragmaticus surrounds the abdominal viscera. Hence, contraction of this muscle tends to squeeze the viscera, rather than simply pushing them caudally and laterally. Without an expansion of the abdominal cavity by the ventral rotation of the pubes, contraction of the diaphragmaticus muscle would increase abdominal pressure immediately. Consequently, during vigorous expiration, abdominal pressure would be elevated as a result of the action of the transversus abdominis and rectus abdominis muscles, and abdominal pressure would remain elevated during inspiration as a result of contraction of the diaphragmaticus muscle. Continuously elevated abdominal pressure may be a significant barrier to venous return from the posterior body (Farmer et al., 1996; Farmer and Hicks, 2000).

Thus, we suspect that some form of active abdominal expansion is necessary for crocodilian diaphragmatic breathing.

Independence of ventilation and locomotion

Alligators have no trouble breathing when they walk. They inspire with large breaths in which air-flow is not interrupted by their locomotion. Indeed, the lack of any limitation on ventilation during walking in these animals is suggested by the exceptionally large air convection requirements that have been measured during locomotion (Farmer and Carrier, 2000). The large costal breaths that occur freely throughout the locomotor cycle contrast sharply with the pattern observed in lizards, in which costal inspiration is disrupted by the locomotor cycle, depressing minute ventilation and gas exchange (Carrier, 1987b; Owerkowitz et al., 1999; Wang et al., 1997). The factors suggested to disrupt costal ventilation in running lizards are also present in walking alligators (albeit to a lesser degree), a semi-erect posture and lateral bending of the trunk. Yet, the breathing pattern of a walking alligator is more reminiscent of the ventilation of a mammal or bird than that of a lizard. How it is that alligators manage to walk and breathe at the same time?

This study suggests that alligators have a number of muscles that are primarily ventilatory in their function. During locomotion, the transversus abdominis, rectus abdominis and ischiopubis muscles exhibited pronounced phasic activity tightly correlated with the ventilatory cycle. In contrast, these muscles displayed only low-level, and often intermittent, activity during locomotion. Furthermore, activity of the diaphragmaticus muscle was never seen to be correlated with the locomotor cycle, but this muscle was always strongly active during inspiration. Thus, alligators have at least four muscles that are primarily or entirely ventilatory in their function. These muscles help to make the breathing of alligators independent of locomotion.

It warrants mention that the primitive function of the rectus abdominis muscle was almost certainly locomotion rather than lung ventilation, and the transversus abdominis muscle is known to play an important role in the locomotion of lizards (Carrier, 1990) and salamanders (Carrier, 1993). Hence, these locomotor muscles appear to have been pre-empted to the function of lung ventilation.

In summary, crocodilians have a highly derived system of lung ventilation: (i) they possess a specialized ventilatory muscle that represents a new invention, the diaphragmaticus muscle; (ii) their rectus abdominis and transversus abdominis muscles, which primitively played an important role in locomotion, now serve a primarily ventilatory function; and (iii) they possess a derived pelvic musculo-skeletal system that appears to be specialized for lung ventilation. This suite of characters is hard to reconcile with the low metabolic requirements and sit-and-wait lifestyle of modern crocodilians. We suspect that these specializations for ventilation reflect strong selection early in the evolution of the crocodilian lineage for a high aerobic activity metabolism and an ability to run and breathe at the same time.

Evolution of pelvic aspiration

The pelvic musculo-skeletal system may have played a role in lung ventilation of the basal archosaurs (Carrier and Farmer, 2000a,b). A form of pelvic aspiration is present in both birds and crocodylians, the two extant members of the archosaur lineage. In some birds, the epaxial muscles contract to rotate the pelvis dorsally during inspiration, and the ventral pelvic musculature and abdominal hypaxial muscles are activated to swing the pelvis ventrally during expiration (Baumel et al., 1990). It is possible that pelvic aspiration evolved independently in the crocodylian and avian lineages. However, given that pelvic aspiration appears to be rare among vertebrates, we believe that some form of pelvic aspiration may have been present in the common ancestor of birds and crocodylians. If this were true, pelvic aspiration arose very early in the archosaur lineage, and the unusual pelvic musculoskeletal system of other archosaurs, such as pterosaurs and dinosaurs, may partially have been shaped by natural selection for the function of lung ventilation.

We thank T. White for conversations and observations that helped lead us to the ventilatory function of the crocodylian pelvic musculoskeletal system. We are grateful to J. W. Hicks, K. Packard, G. Packard and to the Florida Freshwater Fish and Game Commission for supplying us with alligators. We thank Jon Tlachac for his assistance with training the animals. We are indebted to J. W. Hicks for many insights, for helpful comments on the manuscripts and for the use of equipment. Karry Matz provided the artwork for Figs 1 and 6. This study was funded by NSF IBN-9807534 to D.R.C. and NIH 1F32-HL09796-01 to C.G.F.

References

- Ainsworth, D. M., Smith, C. A., Eicker, S. W., Henderson, K. S. and Dempsey, J. A. (1989). The effects of locomotion on respiratory muscle activity in the awake dog. *Respir. Physiol.* **78**, 145–162.
- Baumel, J. J., Wilson, J. A. and Bergren, D. R. (1990). The ventilatory movements of the avian pelvis and tail: function of the muscles of the tail region of the pigeon (*Columba livia*). *J. Exp. Biol.* **151**, 263–277.
- Betts, G., Smith, J. L., Edgerton, V. R. and Collatos, T. C. (1976). Telemetered EMG of fast and slow muscles of the cat. *Brain Res.* **117**, 529–533.
- Boelaert, R. (1942). Sur la physiologie de la respiration de l' *Alligator mississippiensis*. *Arch. Int. Physiol.* **52**, 57–72.
- Carrier, D. R. (1987a). The evolution of locomotor stamina in tetrapods: circumventing a mechanical constraint. *Paleobiology* **13**, 326–341.
- Carrier, D. R. (1987b). Lung ventilation during walking and running in four species of lizards. *Exp. Biol.* **47**, 33–42.
- Carrier, D. R. (1990). Activity of the hypaxial muscles during walking in the lizard *Iguana iguana*. *J. Exp. Biol.* **152**, 453–470.
- Carrier, D. R. (1993). Action of the hypaxial muscles during walking and swimming in the salamander, *Dicamptodon ensatus*. *J. Exp. Biol.* **152**, 453–470.
- Carrier, D. R. (1996). Function of the intercostal muscles in trotting dogs: ventilation or locomotion. *J. Exp. Biol.* **199**, 1455–1465.
- Carrier, D. R. and Farmer, C. G. (2000a). The evolution of pelvic aspiration in archosaurs. *Paleobiology* **26**, 271–293.
- Carrier, D. R. and Farmer, C. G. (2000b). The integration of ventilation and locomotion in archosaurs. *Amer. Zool.*, in press.
- Farmer, C. G. and Carrier, D. R. (2000a). Ventilation and gas exchange during treadmill locomotion in the American alligator (*Alligator mississippiensis*). *J. Exp. Biol.* **203**, 1671–1678.
- Farmer, C. G. and Carrier, D. R. (2000b). Respiration and gas exchange during recovery in alligators. *Respir. Physiol.* **120**, 67–73.
- Farmer, C. G. and Hicks, J. W. (2000). Circulatory impairment induced by exercise in the lizard *Iguana iguana*. *J. Exp. Biol.* **203**, in press.
- Farmer, C. G., Hicks, J. W. and Carrier, D. R. (1996). Effect of the respiratory pump on venous return during exercise in iguanas. *Physiologist* **39**, A103.
- Gans, C. and Clark, B. (1976). Studies on ventilation of *Caiman crocodilus* (Crocodylia: Reptilia). *Respir. Physiol.* **26**, 285–301.
- Huggins, S. E., Parsons, L. C. and Pena, R. V. (1968). Further study of the spontaneous electrical activity of the brain of *Caiman sclerosus*: olfactory lobes. *Physiol. Zool.* **41**, 371–383.
- Loeb, G. E. and Gans, C. (1986). *Electromyography for Experimentalists*. Chicago: University of Chicago Press.
- Maurer, F. (1896). Die ventrale Rumpfmuskulatur einiger Reptilien. *Festschr. Siebenzigsten Geburtstage Carl Gegenbaur Bd.I.*, 184–256.
- Naifeh, K. H., Huggins, S. E. and Hoff, H. E. (1970a). The nature of the nonventilatory period in crocodylian respiration. *Respir. Physiol.* **10**, 338–348.
- Naifeh, K. H., Huggins, S. E. and Hoff, H. E. (1971). The nature of the nonventilatory period in crocodylian respiration. *Respir. Physiol.* **11**, 178–185.
- Naifeh, K. H., Huggins, S. E., Hoff, H. E., Hugg, T. W. and Norton, R. E. (1970b). Respiratory patterns in crocodylian reptiles. *Respir. Physiol.* **9**, 31–42.
- Owerkowicz, T., Farmer, C. G., Hicks, J. W. and Brainerd, E. L. (1999). Contribution of gular pumping to respiration in monitor lizards. *Science* **284**, 1661–1663.
- Romer, A. S. (1923). Crocodylian pelvic muscles and their avian and reptilian homologues. *Bull. Am. Mus. Nat. Hist.* **48**, 533–552.
- Romer, A. S. (1956). *Osteology of the Reptiles*. Chicago: University of Chicago Press. 772pp.
- Wang, T., Carrier, D. R. and Hicks, J. W. (1997). Ventilation and gas exchange during treadmill exercise in lizards. *J. Exp. Biol.* **200**, 2629–2639.
- Youmans, W. B., Murphy, Q. R., Turner, J. K., Davis, L. D., Briggs, D. I. and Hoye, A. S. (1963). *The Abdominal Compression Reaction*. Baltimore: Williams & Wilkins.