Recruitment of the diaphragmaticus, ischiopubis and other respiratory muscles to control pitch and roll in the American alligator (*Alligator mississippiensis*)

T. J. Uriona and C. G. Farmer

Department of Biology, 257 South 1400 East, University of Utah, Salt Lake City, UT 84112, USA

e-mails: uriona@biology.utah.edu; farmer@biology.utah.edu

SUMMARY

We used electromyography on juvenile American alligators to test the hypothesis that the following muscles, which are known to play a role in respiration, are recruited for aquatic locomotion: M. diaphragmaticus, M. ischiopubis, M. rectus abdominis, M. intercostalis internus, and the M. transversus abdominis. We found no activity with locomotion in the transversus. The diaphragmaticus, ischiopubis, rectus abdominis and internal intercostals were active when the animals executed a head-down dive from a horizontal posture. Weights attached to the base of the tail resulted in greater electrical activity of diaphragmaticus, ischiopubis and rectus muscles than when weights were attached to the head, supporting a role of this musculature in locomotion. The diaphragmaticus and rectus abdominis were active unilaterally with rolling maneuvers. Although the function of these muscles in locomotion has previously been unrecognized, these data raise the possibility that the locomotor function arose when Crocodylomorpha assumed a semi-aquatic existence and that the musculoskeletal complex was secondarily recruited to supplement ventilation.

Key words: pitch, roll, aquatic locomotion, lung, alligator, *Alligator mississippiensis*, archosaur, crocodilian, diaphragm, diaphragmaticus, ischiopubis, rectus abdominis, intercostalis, buoyancy, metacentric height, evolution, ventilation, ventilatory mechanics, pelvic aspiration.

INTRODUCTION

Although only semi-aquatic in their habits, crocodilians are adept at aquatic locomotion. They can stealthily adjust their position in the water without a ripple or noticeable muscular activity; they can float passively at the surface or sink and sit comfortably on the bottom. They are remarkable for their ability to vigorously roll about the long axis of their bodies and they glide about the water column with ease (Fish et al., 2007). Maneuverability in water depends upon the generation of lift and drag, two velocity-dependent forces. Thus the ability to maneuver and control posture is much more difficult at slow speeds than at high speeds, a fact that underlies the importance of gas inclusions or lungs to slow swimming organisms (Alexander, 1990; Webb, 2002). Aquatic organisms that have a lung, or some other buoyancy device, can experience a torque if the center of mass and center of buoyancy are not in the same location, and this torque can change the angle of the long axis of the body (a change in pitch) or cause a roll about the long axis.

Whipple (Whipple, 1906) suggested that the musculoskeletal complex of the ypsiloid apparatus of Urodele amphibians functions to shift air in the lungs and thereby change the direction of the long axis of the body in the absence of swimming motions (Whipple, 1906). The basis of this apparatus consists of the cartilago ypsiloides, a γ-shaped cartilage dorsal to the rectus abdominis muscle, the stem of the γ articulating with the pubes in a mobile joint. Whipple hypothesized that contraction of the M. ypsiloideus posterior, a fan-shaped muscle originating on the pubes and inserting on the arms and stem of the cartilage, as well as other muscles associated with the cartilage, pull the ypsiloid cartilage dorsad and thereby squeeze air out of caudal and into cranial portions of the lung, raising the cranial body and lowering the caudal body (Whipple, 1906).

In crocodilians, the part of the musculoskeletal system that enables pelvic aspiration is in many ways remarkably similar to the ypsiloid apparatus. The crocodilian pubes articulate with the ischia in a moveable joint. Fan shaped muscles originating on the ventral, posteriolateral margin of the ischia extend forward and attach to the cranial margin of the pubes, which are expanded paddle-shaped bones completely excluded from the acetabulum. The cranial edges of the pubes are mechanically connected to the gastralia anteriorly through a fibrous sheet and laterally by ligamentous bands (Farmer and Carrier, 2000a). This musculoskeletal complex is mechanically connected with the diaphragmaticus muscle, which originates laterally on the illia, ventrally on the hindmost gastralia and inserts ventrally on the pericardium and a fibrous membrane that encapsulates the liver, and dorsally on an aponeurosis that binds the esophagus to the liver (Reese, 1915). The lungs are mechanically connected to the liver. Thus, contraction of the ischiopubis and diaphragmaticus muscles causes the pubes to rotate ventrocaudad and the viscera to shift ventrocaudad (Farmer and Carrier, 2000a; Gans and Clark, 1976). Although documented to assist ventilation, the liver piston results in a pronounced shift of the lungs and other viscera along the long axis of the body (Brainerd, 1999; Claessens, 2004) (Fig. 1); thus, the muscles and bones that make diaphragmatic and pelvic aspiration possible could plausibly be employed in an aquatic setting to control posture and contribute to maneuverability. If this is true it raises the possibility that the primitive function for this musculoskeletal complex is locomotion, arising when the Crocodylomorpha assumed a more aquatic lifestyle, and that the complex was secondarily recruited for ventilation. Such a scenario implies that the ancestral Archosauromorpha overcame Carrier's constraint (Carrier, 1987) by evolving an upright posture and employing cuirassal breathing (Carrier and Farmer, 2000) rather than

relying on the diaphragmaticus. We used electromyography on juvenile American alligators to test the hypothesis that the following muscles, which are known to play a role in respiration (Farmer and Carrier, 2000a; Gans and Clark, 1976), are recruited for aquatic locomotion: M. diaphragmaticus, M. ischiopubis, M. rectus abdominis, M. intercostalis internus, M. transversus abdominis.

MATERIALS AND METHODS
Experimental design

Five captive raised American alligators (*Alligator mississippiensis* Daudin 1801) were used in this study (0.77–2.2 kg). Surgery was performed on each animal to implant four patch electrodes over different muscles associated with respiration. After implantation of electrodes, the animals were given at least 2 weeks to recover from the surgery in a dry, clean cage before data collection. Following the recovery period, the electrode leads were connected to an AC amplifier (Grass P5, Quincy, MA, USA), and a small inclinometer (2.5 cm×2.5 cm; SQ-SI-360DA SignalQuest, Lebanon, NH, USA) was secured to the back of the alligator just caudal to the scapula. The animal was then placed in a 284-l aquarium (122 cm×45 cm×61 cm; L×W×H) filled three quarters full with 30°C water. The alligator was then given 1 h to adjust to the new environment before data collection commenced. During the observation period, the animals were monitored for muscle activity during three different conditions; with no weight added to the animal, with weight equaling 2.5% of the animal's body mass taped under the jaw, and with weight equaling 2.5% of the animal's body mass on the base of the tail. The order in which the different conditions were monitored was randomly chosen. The period for monitoring muscle activity for each condition lasted for a minimum of 1 h and at least five dives were recorded for each condition. Only dives that had a change in pitch greater than 45° and little change in roll were used for this analysis.

Surgery

Animals were lightly anaesthetized by enclosure in a box containing a rag soaked with isoflurane. Once drowsy, the animals were intubated and ventilated with a small animal ventilator (CWE Inc., Ardmore, PA, USA) using air that had passed through an isoflurane vaporizer (Drager, Lubeck, Germany). The level of anesthesia was initially set at 4%, but was reduced to 0.5% for the majority of the surgery. All surgeries were performed using sterile technique. With the animal in the supine position, the belly and sides were disinfected with betadine and then draped with a sterile cloth. Prior to making the incision, lidocaine was injected just under the skin in the area of the incision. To place electrodes over the diaphragmaticus, the rectus abdominis and the transverses abdominis, an incision was made along the ventral midline of the animal, caudal to the last long rib and cranial to the last gastralia. The ventral portion of the diaphragmaticus muscle was located along both the right and left sides of the body and a patch electrode was secured over the muscle, perpendicular to the muscle fiber orientation. In the same fashion, a patch electrode was sutured in place perpendicular to the muscle fiber orientation on the rectus abdominis and the transverses abdominis muscles. For placement of a patch electrode over the ischiopubis muscle, a small incision was made on the ventral midline of the animal at the location of the hind legs and the patch electrode was placed over the ischiopubis in the same fashion as the above-mentioned muscles. To place a patch electrode over the intercostalis internus a small incision was made perpendicular to the third long rib at the level of the shoulder. The electrode was then placed between the third and fourth long rib between the two layers of
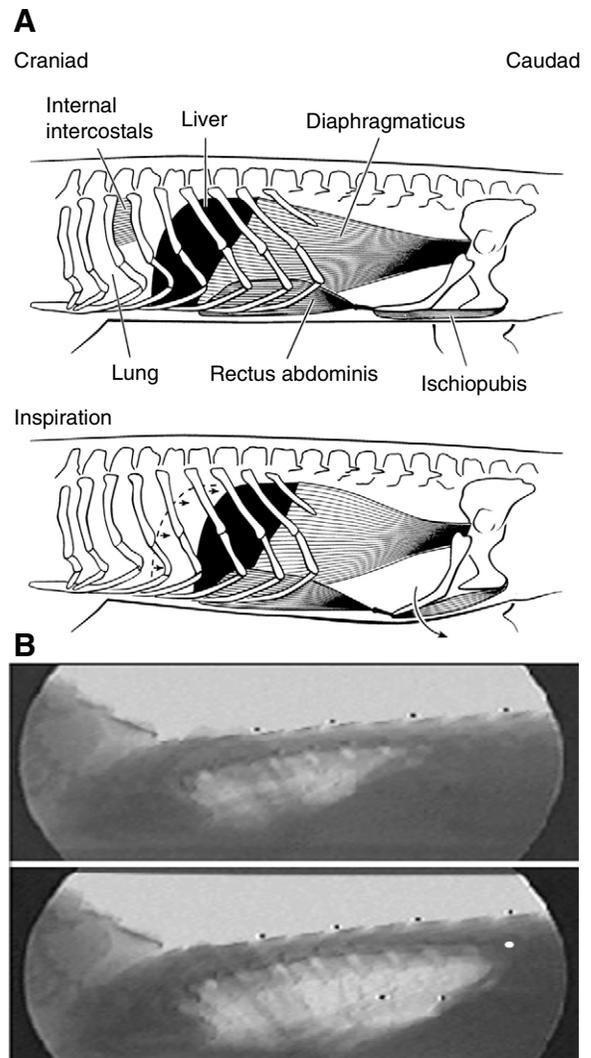

Fig. 1. (A) Illustration of some of the major anatomical features associated with ventilation in crocodilians. Expiration is produced by caudal rotation of the ribs through contraction of the internal intercostals and constriction of the abdominal cavity, which produces a cranial translation of the viscera. Constriction of the abdomen results from activity of the transversus abdominis muscle and the rectus abdominis muscle, which rotate the pubes and gastralia dorsally. Inspiration is produced by cranial rotation of the ribs and caudal translation of the viscera. The viscera, including the large stomach, are pulled caudad by contraction of the diaphragmaticus muscle. The ischiopubis muscles increase the abdominal volume by rotating the pubes and gastralia ventrally. [Diagrams modified with permission from Farmer and Carrier (Farmer and Carrier, 2000a).] (B) X-ray picture illustrating the degree to which the lung is displaced during an inspiration [modified with permission from Brainerd (Brainerd, 1999)].

intercostal muscles and facing the intercostalis internus muscles. A ground electrode was placed inside the abdominal cavity and all electrodes were tunneled out the dorsal side of the body wall. At the exit point, the leads were fed through a rubber tube and the tube was filled with silicone. The leads were anchored against mechanical strain by suturing the rubber tube to the back of the animal. All incisions were sutured and all the animals were treated with a topical antibiotic until the incisions had healed. During healing the animals were housed in a dry enclosure.

Data collection and analysis

Analog signals from the patch electrodes were amplified 5000 times and filtered above 1000 Hz and below 30 Hz. The analog signals from the electrodes and the inclinometer were converted to a digital form using an analog to digital converter (Biopac Systems, Goleta, CA, USA) and stored on a Macintosh computer. Signals were sampled at a rate of 2000 Hz and analyzed with Acqknowledge software (Biopac Systems, Goleta, CA, USA). The muscle activity signals were rectified and analyzed in two ways. First, a dive–surface cycle consisting of a dive underwater immediately followed by a
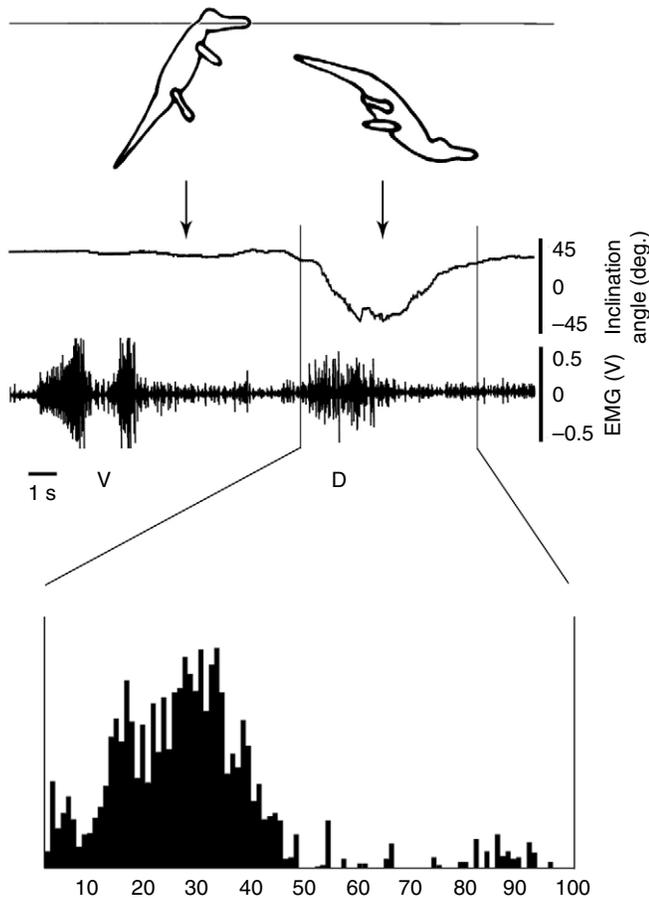

Fig. 2. Sample recordings from alligator 2 (body mass 1.22 kg) of change in pitch (top trace) associated with EMG activity (second trace) in the diaphragmaticus during ventilation and during a dive. The V below the EMG trace indicates muscle activity in the diaphragmaticus associated with inspiration. During this time the alligator's head was level with the water and its body was at a positive 45° angle as illustrated by the schematic alligator above the left side of the pitch angle trace. It should be noted that during this particular ventilation cycle very little change in the pitch angle can be seen. The D below the EMG trace indicates muscle activity in the diaphragmaticus associated with a dive. The vertical bars that mark the beginning of a dive and the subsequent return to the original pitch angle after surfacing from the dive delimit a dive–surface cycle. The maximum pitch angel for this dive–surface cycle was reached at around −45° when the alligator's head was parallel to the surface of the water and its body assumed the indicated angle as illustrated by the schematic (upper right side of the inclinometer trace). In order to compare muscle activity over the course of a dive–surface cycle of different lengths, the cycle was divided into 100 equal bins and the average integrated muscle activity for each bin calculated. A sample of how this was done for a cycle is shown below the EMG trace in this figure. The calibration bar, 1 s.

return to the original pitch angle was divided into one hundred equal bins (Fig. 2). The muscle activity within each bin was then integrated and averaged. For each animal, at least two non-weighted dive cycles were analyzed, and respective bins averaged. The muscle activity within each bin was divided by the average integrated signal for all the bins in order to obtain a relative muscle activity for each bin. For all the animals, the relative muscle activity for respective bins was averaged and the standard error obtained in order to characterize the timing and magnitude of muscle activity for each muscle over the course of a dive–surface cycle (Ritter et al., 2001). Second, muscle activity during dives, for the three treatments, was divided by the change in pitch over the dive in order to get a relative muscle activity for a change in pitch angle. This value was then averaged for all the dives in each treatment. Only dives that had a change in pitch greater than 45° and little change in the roll were used for this analysis. A one-way ANOVA was used to check for a statistically significant treatment effect ($P \le 0.05$).

RESULTS
M. diaphragmaticus

As seen in previous studies, the diaphragmaticus showed phasic activity associated with inspiration when the animals were quietly breathing on land or in the water (Farmer and Carrier, 2000b; Gans and Clark, 1976). Activity in the diaphragmaticus was also observed in a non-respiratory capacity when the animals were diving underwater. Muscle activity was seen during nearly every dive and was always correlated with changes in pitch where the cranial body rotated ventrally along the long axis of the body. The magnitude of muscle activity during these maneuvers was, at times, equal to that observed during quiet breathing at the surface of the water. Most dives were preceded by an inspiration, whereupon the diaphragmaticus remained active until the maximum difference in pitch angle was reached (at around 50% of the dive–surface cycle). Under this condition, the maximum average muscle intensity was reached at about 20% of a dive–surface cycle. When all the dives for each animal were averaged together the beginning of muscle activity associated with diving was obscured by the ventilation activity that preceded the dive (Fig. 3). When a breath did not precede a dive, again the muscle only remained active until the maximum difference in pitch angle was reached. However, a full cycle of muscle activity was observed, and the maximum average muscle intensity was reached slightly later than that obtained in the cycles preceded by a breath (example of this can be seen in Fig. 2). When the animals were weighted with 2.5% of their body mass under the jaw, the average integrated area of the electromyographic (EMG) signal for a given change in pitch during a dive was significantly smaller then when the same weight was placed on the base of the tail. This was true for all but one of the animals. When the weight was placed on the head of this animal it would not make a head-down dive and would sink with the body parallel to the horizontal plane. However, for this animal the average integrated area of the EMG signal for a given change in pitch during a dive was significantly larger when the weight was placed on the tail than when no weight was added to the animal. In general, the activity of the diaphragmaticus was greatest for a given change in pitch when the weight was placed on the tail and least when the weight was placed under the jaw, with the trials where no weight was added falling between the other two (Fig. 4). We also observed that the diaphragmaticus had the ability to become active differentially on either side of the body. If the animal were to roll hard to one side during a dive, the muscle on the side leading the roll would become active, whereas activity on the opposite side could be seen to diminish or stop (Fig. 5).

A   Diaphragmaticus

B   Ischiopubis

C   Rectus abdominis

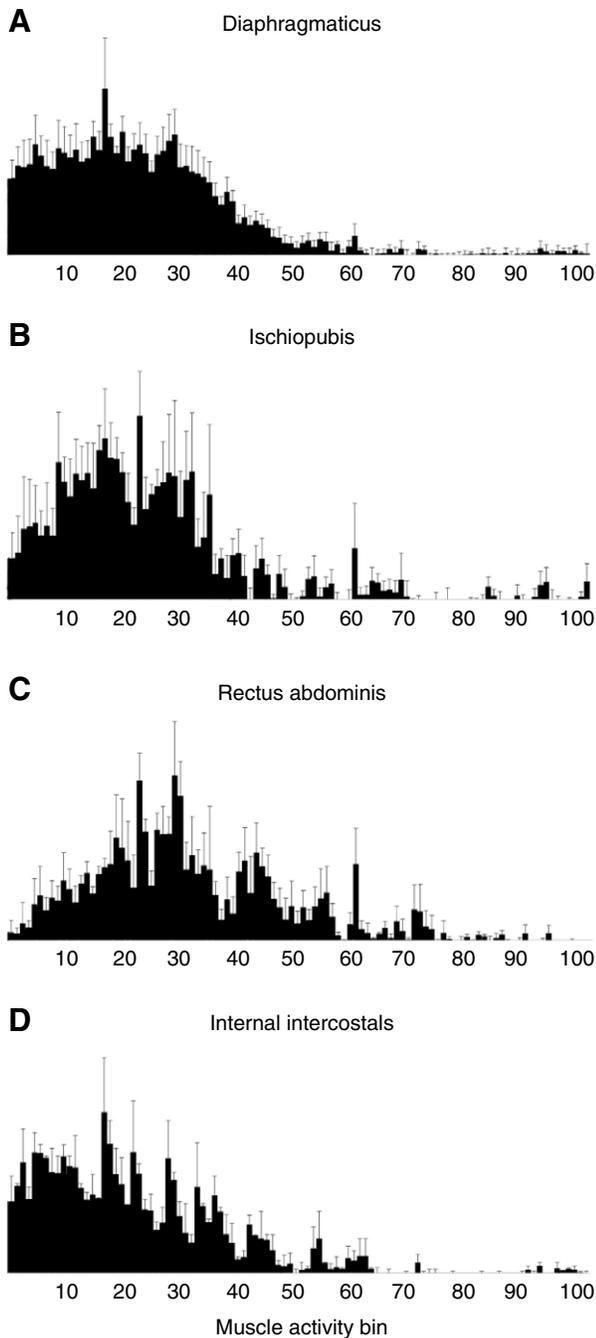**D**   Internal intercostals

Muscle activity bin

Fig. 3. Graphs of timing and intensity of muscle activity during a dive–surface cycle with the relative muscle activity of each muscle divided into 100 bins and expressed as means ± s.e.m. for each bin. (A) Diaphragmaticus, $N=7$; (B) ischiopubis; $N=4$; (C) rectus abdominis, $N=4$; (D) internal intercostals, $N=2$.

M. ischiopubis

Like the diaphragmaticus, activity in the ischiopubis was consistent with that observed in other studies, with the muscle showing phasic activity associated with quiet ventilation on land and in the water (Farmer and Carrier, 2000a). However, the magnitude of activity was greatly reduced, or at times absent during ventilation in the water. The ischiopubis was also like the diaphragmaticus in that the muscle showed activity associated with the animal diving underwater.

Again, muscle activity was seen during almost every dive and was always correlated with changes in pitch, which rotated the cranial body of the animal ventrally. The magnitude of muscle activity during these maneuvers was greater than that observed during quiet breathing at the surface of the water. Because of this, the initial average activity level for this muscle during the start of a dive was reduced in relation to the maximum intensity, despite the fact that most of the dives were preceded by an inspiration. The ischiopubis remained active for less time than the diaphragmaticus and finished firing at about 45% of a dive–surface cycle. The maximum average muscle intensity for all the animals showed two peaks at about 17 and 25% of a dive–surface cycle (Fig. 3). When the animals were weighted under the jaw and on the tail, the same general trends were observed for the average integrated EMG activity for a given change in pitch as that observed for the diaphragmaticus. A statistically significant treatment effect was observed when comparing the values for weight under the jaw with weight added to the tail, with the largest values observed when the weight was on the tail (Fig. 4).

M. rectus abdominis

As reported previously, the rectus abdominis was active during exhalation when breathing on land (Farmer and Carrier, 2000a). However, in this study it was never active during exhalation while the animal was breathing on the surface of the water. This muscle, like the diaphragmaticus and the ischiopubis, was active when the animal dived underwater. Activity was seen during almost every dive and was always correlated with changes in pitch, which rotated the cranial body of the animal ventrally. The muscle became active at the start of a dive and the maximum intensity was reached around 30% of the dive–surface cycle. The muscle remained active for a longer period than that observed with the *diaphragmaticus* or *ischiopubis*, and was finished firing at about 55% of a dive–surface cycle (Fig. 3). When the animals were weighted under the jaw and on the tail, the same trends were observed for the average integrated EMG activity for a given change in pitch as was observed for the diaphragmaticus and ischiopubis. A statistically significant treatment effect was observed when comparing the values for weight under the jaw with weight added to the tail, with the largest values observed when the weight was on the tail (Fig. 4). Like the diaphragmaticus, the rectus abdominis appears to become active independently on each side during a dive when the animal was rolling to one side or the other (Fig. 5).

M. intercostalis internus

Activity in the intercostalis internus muscle was observed during exhalation when the animal was on land. However, this muscle was not active during exhalation when the animal was breathing on the surface of the water. The internal intercostals showed activity associated with the animal diving. Muscle activity was seen during almost every dive and was always correlated with a change in pitch in which the cranial body rotated ventrally. Activity in this muscle was initiated before the start of a dive, reached its maximal average intensity between 10 and 20% of the dive cycle, and was quieted by 60% of a dive–surface cycle (Fig. 3).

M. transversus abdominis

Like the rectus abdominis and in accordance with what has been previously reported, this muscle was active during exhalation when the animal was on land (Farmer and Carrier, 2000a). However, this muscle was inactive during exhalation when the animal was breathing at the surface of the water. This muscle showed no activity when the animals were diving or surfacing from a dive.
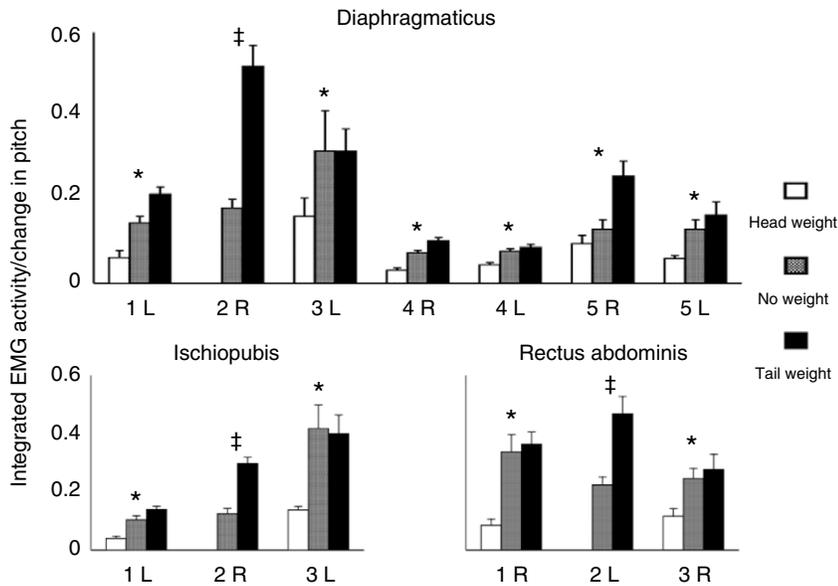
Fig. 4. Graphs of the integrated EMG activity divided by change in pitch (mean ± s.e.m.) in the diaphragmaticus, ischiopubis and rectus abdominis muscles for each alligator during a dive under three conditions; with no weight added to the animal (gray bars), with weight equaling 2.5% of the animal's body mass under the jaw (white bars), and with weight equaling 2.5% of the animal's body mass on the base of the tail (black bars). Numbers 1–5 indicate the alligator that was used, followed by a L or R, which indicates if the muscle on the left or right side, respectively, was used; *significant treatment effect when comparing head weighted to tail weighted dives ($P \leq 0.05$); ‡significant treatment effect when comparing dives where no weighted was added to tail weighted dives ($P \leq 0.05$). The values for integrated EMG activity divided by the change in pitch should not be compared between animals or muscles because of differences in electrodes, placement of electrodes, and individual muscle activity.

DISCUSSION

Ventilatory mechanics of crocodilians consist of costal ventilation, in which the external intercostal muscles change the volume of the thorax by swinging the ribs laterocraniad during inspiration and the internal intercostal muscles swing the ribs mediocaudad during exhalation (Gans and Clark, 1976). In addition, inspiration is facilitated when ischiopubis muscles rotate the pubes and the gastralia caudoventrad while the diaphragmaticus muscle pulls the liver–lung complex caudad (Farmer and Carrier, 2000a; Gans and Clark, 1976). The rectus abdominis and transversus abdominis contract during exhalation to squeeze the liver and other viscera craniad (Farmer and Carrier, 2000a; Gans, 1976). With large breaths, the liver–lung complex undergoes very large craniocaudal

displacements (Brainerd, 1999; Claessens, 2004). On land this form of breathing shifts the center of mass largely fore and aft (personal observations). However, in water, movement of the liver-piston will move the center of buoyancy as well as the center of mass.

The relative position of the center of mass and the center of buoyancy is a principle determinant of the stability and maneuverability of the organism (Webb, 2002). A torque will be exerted along the long axis of the body if the center of buoyancy is either cranial or dorsal to the center of mass. Similarly, if the center of buoyancy is ventral to the center of mass the animal can easily roll. Although a highly maneuverable design, this situation is also unstable (Webb, 2002). By shifting the center of buoyancy
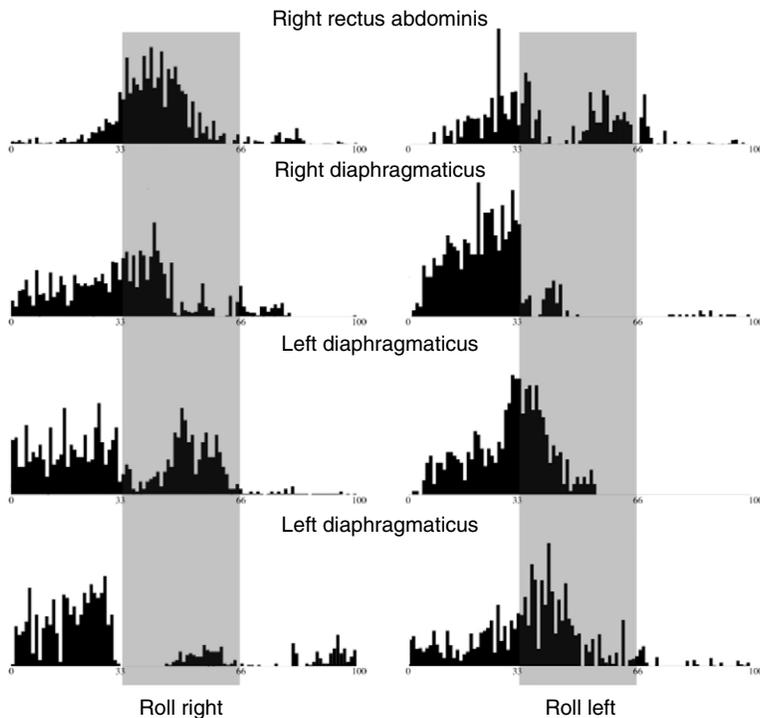

Fig. 5. Sample data for two alligators showing timing and intensity of muscle activity as the average integrated EMG activity divided into 100 bins during a dive where the animals rolled hard to the right side (clockwise) and to the left side (counterclockwise). The shaded area in the middle of the graphs represents the period of time during the dive where the animals rolled to the right or the left side respectively. An equal period prior to and following the period of time associated with the roll is shown on either end of the graphs. The first set of graphs shows muscle activity in the right rectus abdominis of alligator 2. The second set of graphs shows muscle activity in the right diaphragmaticus of alligator 5. The third set of graphs shows muscle activity in the left diaphragmaticus of alligator 5. The final set of graphs shows muscle activity in the left diaphragmaticus of alligator 2. For both animals muscle activity can be seen to diminish or stop in left diaphragmaticus during the period of time that the animal was rolling to the right and the opposite is seen as the animal rolls to the left. The same trend can be seen in the right rectus abdominis in alligator 2 and the right diaphragmaticus in alligator 5. At around 66% of the entire cycle the animal is no longer diving down and muscle activity stops for all the muscles shown in both alligators.

with respect to the center of mass, crocodilians could control posture, pitch and rolls. Thus we analyzed the activity of several muscles that have the capacity to shift the center of buoyancy.

When pivoting about the long axis of the body so that the head tilts down and the tail up, we measured electrical activity in the diaphragmaticus, the ischiopubis, the rectus abdominis and the internal intercostals. Although the rectus abdominis and the intercostal muscles are important in movements of the trunk in lizards (Carrier, 1990), the ischiopubis and diaphragmaticus have little if any mechanical ability to bend the trunk. During exercise on land the transversus abdominis, rectus abdominis and ischiopubis muscles have only low-level, and often intermittent activity during locomotion but they have a strong phasic pattern of activity tightly correlated with ventilation (Farmer and Carrier, 2000a). During ventilation the diaphragmaticus and ischiopubis muscles move the lung ventrocaudad. Therefore, we think that when these muscles are employed in an aquatic setting they increase the relative buoyancy of the caudal body while decreasing the relative buoyancy of the cranial body, and thereby change pitch. Furthermore, when we made this maneuver more difficult by adding a weight to the base of the tail, or easier by adding a weight to the head, the activity of these muscles changed in a manner that is consistent with the hypothesis, that is, more activity was measured when the weight was on the tail than when the head was weighted.

With rolling maneuvers we measured differences in the levels of activity of the right and left diaphragmaticus and rectus abdominis muscles. When the animals rolled clockwise around the long axis of rotation (the right side of the body moving ventrad) activity in the right moiety of these muscles increased whereas activity in the left moiety decreased. A counterclockwise roll was accompanied by an increase in the activity of the left moieties and a decrease in the right moieties (see Fig. 5). Prior to this study we know of no studies showing unilateral activity in the right and left side of the diaphragmaticus. This high degree of neural sophistication associated with the ability to control ones position from side to side in water further highlights the importance of this muscle to aquatic locomotion.

Evolutionary implications

The evolutionary history of the breathing mechanisms of amniotes is a central chapter in what is arguably one of the most exciting stories in the history of life, the water–land transition. The common ancestor of tetrapods used the mouth, the buccal pump, to ventilate both gills and lungs (Gans, 1970). The rise of novel breathing mechanisms that eventually replaced buccal ventilation has long been of interest (Brainerd, 1999; Perry et al., 2005). How and when did novel respiratory muscles, such as the diaphragmaticus, evolve? Our results may provide new insight into the origin of the crocodilian diaphragmaticus in offering a plausible and logical scenario for the sequence of events giving rise to this new muscle.

We observed that alligators activate the rectus abdominis and the diaphragmaticus in synchrony when diving in water, despite the fact that the rectus abdominis is used for exhalation and the diaphragmaticus for inhalation during ventilation. Both the rectus abdominis and the diaphragmaticus were probably originally derived from the same muscle-group in alligators (Carrier and Farmer, 2000; Keith, 1905) and the primitive function of the rectus muscle was almost certainly locomotion rather than ventilation. To have a favorable function in aquatic locomotion just one innovation would have been required, a change of the site of insertion of a portion of the rectus from the sternum to the liver. By contrast, to evolve this muscle for the purpose of respiration requires two evolutionary

innovations to occur, a deviation of the site of insertion of part of the rectus from the sternum to the liver and the development of new motor recruitment patterns. Thus, the most parsimonius explanation for the origin of the diaphragmaticus is that it arose first for controlling movement in the water and was later recruited for ventilation.

Early crocodilians appear to have been fully terrestrial animals. In the Triassic period (approximately 245 million years ago) they were small (about the size of a cat), slender animals with traits indicative of a capacity for agile locomotion, such as a parasagittal limb posture and reduced numbers of digits on long slender limbs (Parrish, 1987; Walker, 1970). It is in the Cretaceous (roughly 145 million years ago) that the lineage became amphibious and acquired many features associated with a life in water (e.g. the dorsoventrally flattened skull, short limbs and expanded tail) (Sill, 1968). Although it has been suggested that both cuirassal inspiration and a diaphragmaticus supplemented costal inspiration in the Triassic forms (Carrier and Farmer, 2000; Farmer and Carrier, 2000a), the data of this study support the origin of this muscle in the Cretaceous with the assumption of an aquatic lifestyle (Paul, 2002). If this is true, then cuirassal breathing and upright posture may have been the primary mechanisms by which early archosaurs overcame Carrier's constraint on simultaneous costal ventilation and locomotion (Carrier, 1987).

Inferences of the evolutionary history of the crocodilian diaphragmaticus will be strengthened with further studies of similar muscles that have been convergently acquired because convergent evolution is one of the most robust lines of evidence for the adaptive significance of traits. Diaphragm-like muscles have been identified in some frogs (*Xenopus laevis* and *Pipa pipa*) (Keith, 1905; Snapper et al., 1974), and some testudines (e.g. *Trionix*) (Gaunt and Gans, 1969; George and Shah, 1959) and prior studies implicate a respiratory or gastrointestinal function in these lineages (Pickering et al., 2004; Pickering and Jones, 2002; Snapper et al., 1974). However, electrical activity of the amphibian diaphragm suggests a role for this muscle in aquatic locomotion (De Jongh, 1972) and we have measured electrical activity in the chelonian diaphragmaticus that is coincident with changes in pitch and roll during aquatic locomotion (T.J.U. and C.G.F., personal observations). The anuran diaphragm has no relationship to the ypsiloid apparatus of salamanders, which is yet again an independently evolved but analogous mechanism for adjusting the center of buoyancy (Whipple, 1906). Finally, the diaphragms of Sirenia are highly derived in a way that could allow the lungs to be used to control pitch and roll (Rommel and Reynolds, 2000). Thus, there may be at least six examples of innovations that enable the gases in the lungs to be manipulated to control posture, pitch and roll in tetrapods, suggesting there is strong positive selection for this function. We find this ability to be quite remarkable, with implications related to aquatic locomotion in many vertebrates that possess a lung or gas bladder.

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