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MECHANICS OF LUNG VENTILATION IN A LARVAL SALAMANDER *AMBYSTOMA TIGRINUM*

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Summary

The larval stage of the tiger salamander *Ambystoma tigrinum* is entirely aquatic, but the larvae rely on their lungs for a large proportion of their oxygen uptake. X-ray video and pressure measurements from the buccal and body cavities demonstrate that the larvae inspire using a two-stroke buccal pump and exhale actively by contracting the hypaxial musculature to increase body pressure. Larvae begin a breath by expanding the buccal cavity to draw in air through the mouth, while simultaneously exhaling air from the lungs to mix with the fresh air in the buccal cavity. The mouth then closes, and the buccal cavity compresses to pump a portion of the mixture into the lungs. The remaining air in the buccal cavity is then released as bubbles from the mouth and gill slits. Ventilatory volumes estimated from X-ray video records indicate that approximately 80 % of the air pumped into the lungs is fresh air and 20 % is previously expired air. Exhalation in larval tiger salamanders is active, powered by contraction of all four layers of lateral hypaxial musculature. Electromyography indicates that the transverse abdominis (TA) muscle is active for the longest duration and shows the highest-amplitude activity, but the external oblique superficialis, the external oblique profundus and the internal oblique also show consistent, low-level activity. The finding that the TA muscle is active during exhalation in larval tiger salamanders contributes to a growing body of evidence that the use of the TA for exhalation is a primitive character for tetrapods.

Key words: respiration, aspiration, buccal pump, pulse pump, exhalation, evolution, physiology, Amphibia, Lissamphibia, Urodela, hypaxial muscle, salamander, *Ambystoma tigrinum*.

Introduction

The larvae of the tiger salamander *Ambystoma tigrinum* live entirely in water until their metamorphosis into terrestrial adults. The larvae exhibit several features which specialize them for obtaining oxygen from water, such as external gills and thin, highly vascularized skin. Even with these features, however, larval tiger salamanders rely on their lungs for a large proportion of the total oxygen required. Previous studies have found that 40–60 % of the oxygen is obtained through the lungs when the larvae are in normoxic water at temperatures ranging from 15 to 25 °C (Whitford and Sherman, 1968; Heath, 1976). In hypoxic water at 23 °C, 70 % of the oxygen is obtained from the lungs (Heath, 1976).

Little is known about the lung ventilation mechanism of larval tiger salamanders. Superficial observations have shown that they inspire using a buccal pump (Whitford and Sherman, 1968), but nothing is known about the pattern of air transfer between the atmosphere, buccal cavity and lungs, or about the mechanics of exhalation. Lung ventilation in salamanders has been investigated in only three species, *Siren lacertina*, *Amphiuma tridactylum* and *Necturus maculosus* (Martin and Hutchison, 1979; Brainerd *et al.* 1993; Brainerd and Monroy, 1998), although the literature on gas exchange during bimodal and trimodal breathing in salamanders is extensive (e.g. Whitford and Sherman, 1968; Toews, 1973; Guimond and Hutchison, 1976; Heath, 1976; Boutilier *et al.* 1980; Burggren and Wood, 1981; Ultsch and Duke, 1990).

Previous studies of respiratory mechanics in amphibians and lungfishes have revealed a shared pattern of gas transfer known as a two-stroke buccal pump (Brainerd *et al.* 1993; Brainerd, 1994). Two-stroke breathing has been observed in lungfishes (Bishop and Foxon, 1968; McMahon, 1969), frogs (e.g. de Jongh and Gans, 1969; Wang, 1994), salamanders (Brainerd *et al.* 1993; Brainerd and Monroy, 1998) and a caecilian (Carrier and Wake, 1995). In two-stroke breathing, both exhalation and inhalation occur within one cycle of buccal expansion and compression (named by analogy with a two-stroke engine). Because the buccal cavity is not emptied after exhalation, some of the expired gas is mixed with fresh air in the buccal cavity and pumped back into the lungs. In contrast to the two-stroke buccal pump, many air-breathing fishes and a few amphibians use a four-stroke buccal pump (Brainerd *et al.* 1993; Brainerd, 1994). In four-stroke breathing, the buccal cavity compresses

and empties completely after exhalation, and then expands and compresses for a second time to pump fresh, unmixed air into the lungs. Four-stroke breathing is found in basal, ray-finned fishes, such as *Amia* and *Lepisosteus*, in two salamanders (*Amphiuma tridactylum*, Brainerd *et al.* 1993; *Cryptobranchus alleganiensis*; E. L. Brainerd, personal observation) and one frog (*Xenopus laevis*; Brett and Shelton, 1979).

For the purpose of comparing different breathing mechanisms, a 'lung ventilation efficiency' (LVE) is defined here as the percentage of fresh air that is pumped into the lungs with each breath. The LVE of a four-stroke buccal pump is 100 % because only fresh air is pumped into the lungs. The LVE of a two-stroke pump is expected to be less than 100 % because some expired air is rebreathed. A primary goal of this study is to determine whether larval tiger salamanders use a two-stroke or a four-stroke buccal pump and to measure their LVE using estimates of lung and buccal cavity volumes from X-ray video records of breathing.

A second goal of this study is to determine whether larval tiger salamanders exhale actively or passively. Passive exhalation is generally driven by elastic recoil of the lungs and body wall (Liem, 1985). In aquatic animals, hydrostatic pressure also contributes to passive exhalation (Gans, 1970). Passive exhalation has been found in ray-finned fishes, lungfishes and caecilians (McMahon, 1969; Deyst and Liem, 1985; Carrier and Wake, 1995). In contrast to passive exhalation, active exhalation is produced by the contraction of the hypaxial musculature surrounding the body cavity, thus increasing body cavity pressure and driving air from the lungs. Previous work on two salamander species, *Siren lacertina* and *Necturus maculosus*, showed that these salamanders exhale actively by contracting their lateral hypaxial musculature, primarily the transverse abdominis muscle (Brainerd *et al.* 1993; Brainerd and Monroy, 1998). If the transverse abdominis is also found to be active during exhalation in larval tiger salamanders, then this result would contribute to a growing body of evidence that the use of the transverse abdominis for exhalation is a primitive character for salamanders and perhaps for all tetrapods (Brainerd *et al.* 1993; Brainerd and Monroy, 1998).

Materials and methods

Animals and recording conditions

Premetamorphic *Ambystoma tigrinum* (Greene) were obtained from a scientific supplier (Sullivan's Amphibians, Nashville, TN, USA) and maintained in water in a cold room (15 °C) in order to prevent metamorphosis. The largest possible specimens were obtained; the 12 specimens used for physiological recordings had snout–vent lengths of 11.5–12.6 cm. The animals were maintained on a diet of earthworms and were not fed for 2 days prior to experiments. All measurements were performed in air-equilibrated water at room temperature (21–23 °C), and animals were removed from the cold room and allowed to acclimate to room temperature for at least 24 h before recordings began. At room temperature, the animals breathed air regularly, approximately one breath every 2–5 min, and thus it was not necessary to deoxygenate the water in order to induce air-breathing behavior.

Observations of air-breathing behavior in larval *A. tigrinum* showed that, in water deeper than 6 cm, the animals had to swim to the surface to breathe. In shallower water, they had to bend their backs in a lordotic curve to raise their mouths above the surface of the water. Thus, all measurements were performed in water 6 cm deep.

X-ray videography

X-ray videography was used to observe the pattern of air flow between the atmosphere, buccal cavity and lungs. Both lateral and dorsoventral views were recorded from three individuals. From two individuals, five lateral and five dorsoventral breaths were analyzed, and from the third individual, six lateral and three dorsoventral breaths were analyzed. A Siemens cineradiographic unit with a Sirecon image intensifier and a Sony VX1000 digital camcorder (shutter speed 1/250 s) were used to record X-ray videos at a time resolution of 60 fields s^{-1}.

For kinematic analysis, video sequences were digitized with a Radius Video Vision Studio computer board in a Macintosh computer. Video clips were deinterlaced and converted to numbered PICT files at 60 fields s^{-1}. The PICT files were opened in NIH Image, and the projected areas of the buccal cavity and of one lung were traced and measured. The plots of area *versus* time were then averaged for the number of breaths collected (3–6 breaths in lateral and dorsoventral views per individual).

Estimation of ventilation volumes

Mean lateral and dorsoventral areas were combined to estimate the tidal volumes of the lungs and buccal cavity. The lungs were modeled as elliptical cylinders. The length (l) of the lungs was measured for each sequence and a mean value was calculated. Dividing the mean dorsoventral and lateral areas by the length of the lungs yielded mean values for the major (x) and minor (y) axes of the ellipse. The volume (V) of the elliptical cylinder was then calculated for each field of video as $V=\pi l(x/2)(y/2)$. Buccal volume could not be modeled by a regular geometric shape. Instead, the mean lateral area of the buccal cavity in each field was multiplied by the mean dorsoventral area and then divided by the length of the buccal cavity. This yielded an irregular shape that takes into account the lateral and dorsoventral areas of the buccal cavity, but has square rather than rounded corners. The square corners may cause a slight overestimate of buccal volume.

The volume estimates were used to calculate the change in buccal volume (V_b), expired volume (V_e), inspired volume (V_i) and residual volume of air in the lungs after exhalation. For each animal, a lung ventilation efficiency (LVE) was calculated as $(V_b–V_e)/V_b$, and the proportion of buccal volume pumped into the lungs was calculated as V_i/V_b.

Unfortunately, it is not clear how these volumes might be verified by direct measurements of flow. The most common

technique for measuring ventilation volumes in aquatic air-breathers is blowhole pneumotachography (e.g. Boutilier, 1984; Vitalis and Shelton, 1990). This technique cannot be used for mouth-breathing salamanders, such as larval tiger salamanders, because they thrust their snout above the surface when they breathe (see Fig. 1). In a blowhole system, the snout displaces air in the blowhole chamber, thus producing air-flow artifacts that overwhelm the air-flow signals due to breathing. Furthermore, in animals that use a two-stroke buccal pump, measuring air flow at the mouth or nares yields values for the change in buccal volume, but this technique does not measure the tidal volume of the lungs (although any net inflation or deflation of the lungs can be calculated). Thus, although the radiographic technique used in the present study only approximates mean air flow over several breaths, it is probably the best method for estimating volumes and flow rates in larval *A. tigrinum.*

Pressure recordings

Buccal and pleuroperitoneal cavity pressures were measured as described by Brainerd *et al.* (1993). Salamanders were anesthetized in a solution (1 g l^{-1}) of tricaine methanesulfonate (Finquel brand, Argent), buffered to pH 7 with sodium bicarbonate. A small hole was drilled in the snout of the animal, and a polyethylene cannula (1.27 mm o.d., PE 90) was introduced into the buccal cavity. A 14 gauge hypodermic needle was used to introduce a similar cannula into the pleuroperitoneal cavity, outside the lungs. We began to measure pressures 1–3 h after surgery, and continued to make measurements for 24–48 h. No difference was noted between shorter and longer recovery times.

For recording pressures, Millar Microtip SPR-407 pressure transducers were threaded down the cannulae. Pressures were amplified 100× with Tektronics AM502 direct current amplifiers and recorded on a Macintosh computer with a GW Instruments data acquisition board and SuperScope II software. Pressure waves were digitized at 50 samples s^{-1}.

Color-key graphics overlay (Televeyes Pro, Computer Eyes, Inc.) was used to synchronize pressure waves with standard and X-ray video recordings. Real-time pressure traces from SuperScope II were superimposed onto live video recordings of animals breathing. The combined, synchronized images were then recorded on video cassette for analysis.

Electromyography

Four specimens were dissected to determine the muscle fiber orientations of the four layers of lateral hypaxial musculature: external oblique superficialis (EOS), external oblique profundus (EOP), internal oblique (IO) and transverse abdominis (TA). Fiber angles were measured for ten muscle fibers per myomere and two myomeres per individual.

Patch electrodes were used to minimize cross-talk between the thin sheets of lateral hypaxial musculature (Loeb and Gans, 1986; Carrier, 1990). Recordings were made from the four layers of lateral hypaxial musculature and from the rectus abdominis (RA). Patch electrodes were constructed from silastic sheeting (Dow Corning, 0.25 mm thick), fine wire (stainless steel, 0.051 mm diameter) and liquid silicone. Electrodes were manufactured and implanted as described by Carrier (1993). The TA electrode was placed between the TA and IO, with the recording side facing the TA. The IO electrode was placed in the same location, facing the IO. The EOS electrode was placed between the EOS and EOP, facing the EOS; the EOP electrode was placed in the same location, facing the EOP.

Electromyograms (EMGs) were amplified 10 000× with Grass Instruments P511J amplifiers, set to a bandpass of 30 Hz to 10 kHz, with a 60 Hz band-stop filter. Most EMG signals were digitized at 2000 points s^{-1}, but EMGs from one individual were digitized at 4000 points s^{-1}. Power spectrum density analysis of signals recorded at 4000 points s^{-1} showed the main power of EMG frequencies to be in the range 100–500 Hz, indicating that a sampling rate of 2000 points s^{-1} was sufficient to capture the onset and duration of the EMG signals (Jayne *et al.* 1990). This sampling rate may not have been sufficient, however, to capture absolute amplitude information (Moon, 1996). The relative amplitudes of the EMG signals should, however, be indicative of the relative amplitudes of the analog signals.

Pleuroperitoneal pressures were recorded simultaneously with EMG signals, and all signals were synchronized with standard video recordings to confirm normal breathing behavior for each breath.

Quantitative analysis of pressures and EMGs

Two pressure magnitudes and four timing variables were measured from the buccal and body pressure traces of at least 20 breaths from four individuals: P_{body}, the magnitude of body pressure; P_{buccal}, the magnitude of buccal pressure; T_{body}, the time from the beginning of mouth opening to peak body pressure; T_{gape}, the duration of open gape; T_{buccal}, the time from the beginning of mouth opening to peak buccal pressure; and T_{total}, the total time required for lung inflation. Shapiro–Wilk *W*-tests detected no deviation from normality in the variables within each individual ($P>0.05$ for all variables), and thus a one-way analysis of variance (ANOVA) was used to test for significant differences between individuals.

Two EMG variables were measured from each of the five muscles studied: the onset of activity relative to the beginning of pressure increase in the body cavity, and the duration of activity. Shapiro–Wilk *W*-tests detected no deviation from normality in these variables ($P>0.05$ for all variables); a one-way ANOVA was therefore used to test for significant difference between individuals. In addition, a nested ANOVA (individuals nested within experiment type) was used to test for significant differences in body pressure magnitude between EMG experiments and experiments in which only buccal and body pressures were measured.

Results

Kinematics

The pattern of air transfer between the atmosphere, the buccal cavity and the lungs is shown in still fields from a

lateral-view X-ray video of lung ventilation in a larval *Ambystoma tigrinum* (Fig. 1). Measurements of buccal and lung areas in lateral projection for six breaths from one individual quantify the pattern of air transfer (Fig. 2). Lung ventilation starts as the animal approaches the surface and begins to open the mouth and expand the buccal cavity (note that larval tiger salamanders are mouth-breathers, whereas the adults breathe through nares). Exhalation begins while the buccal area is still expanding and continues for approximately 0.1 s. A small amount of air remains in the lungs at the end of exhalation. Peak buccal expansion occurs 30 ms after peak mouth gape, and a pause then ensues during which the buccal cavity remains full of air but no air is pumped into the lungs (Fig. 2, 0.12–0.25 s). Buccal compression then forces air into the lungs, and the lungs are full by 0.40 s. After the lungs have been filled, some air remains in the buccal cavity; this air exits the buccal cavity first from the gill slits (Fig. 1, 0.43 s) and then from the mouth (Figs 1, 2, 0.50 s).

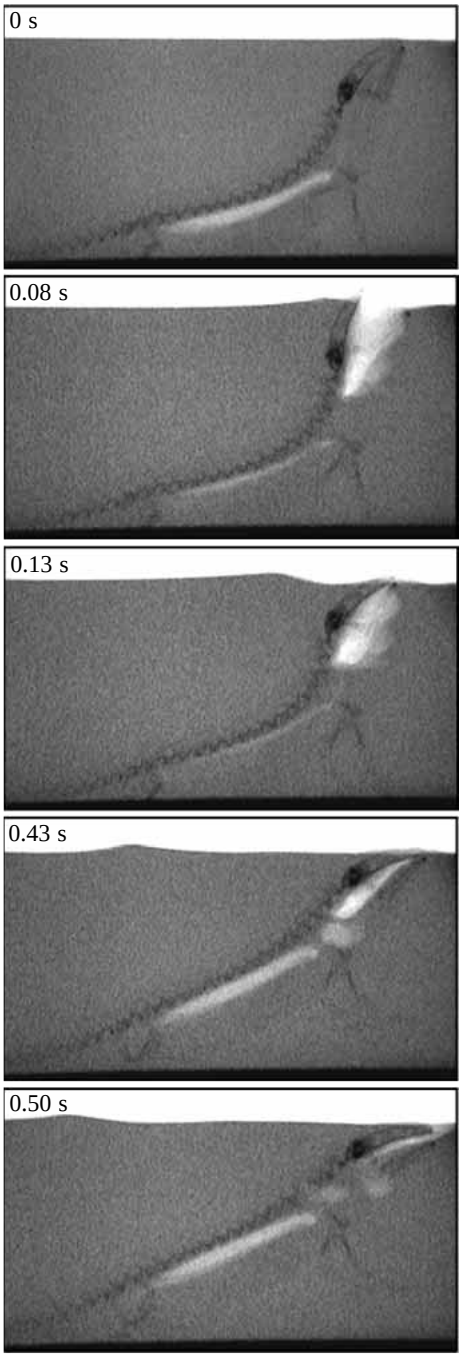

Fig. 1. Still fields from an X-ray video record of lung ventilation in a larval *Ambystoma tigrinum*, lateral view. The fields are printed as X-ray positive images to emphasize the light-colored air contained in the lungs and buccal cavity. The animal is immersed in water (gray background) and lifts its mouth to breathe above the surface (the white strip at the top of the fields). Between 0 and 0.08 s, the buccal cavity fills with air and exhalation occurs. Between 0.13 and 0.43 s, the buccal cavity compresses, filling the lungs with air, and extra air exits as bubbles from the gill slits. At 0.50 s, the remaining air in the buccal cavity exits from the mouth.

In dorsoventral projection, both lungs can be seen, as well as the shape of the expanded buccal cavity (Fig. 3). Measurements of the dorsoventral area of the buccal cavity show that the maximum dorsoventral area is attained within two frames after the beginning of mouth opening (Fig. 4) and that the area remains near this maximum value until air is released from the mouth at the end of the breath (Fig. 3, 0.55 s). For comparison with the lateral area in Fig. 2, the dorsoventral area of only one lung is shown in Fig. 4. For the three individuals studied, the mean maximum area changes were: lateral lung, 1.5±0.09 cm^2; dorsoventral lung, 1.2±0.21 cm^2; lateral buccal cavity, 2.6±0.0.20 cm^2; dorsoventral buccal cavity, 4.6±0.05 cm^2 (means ± S.E.M., N=3 individuals).

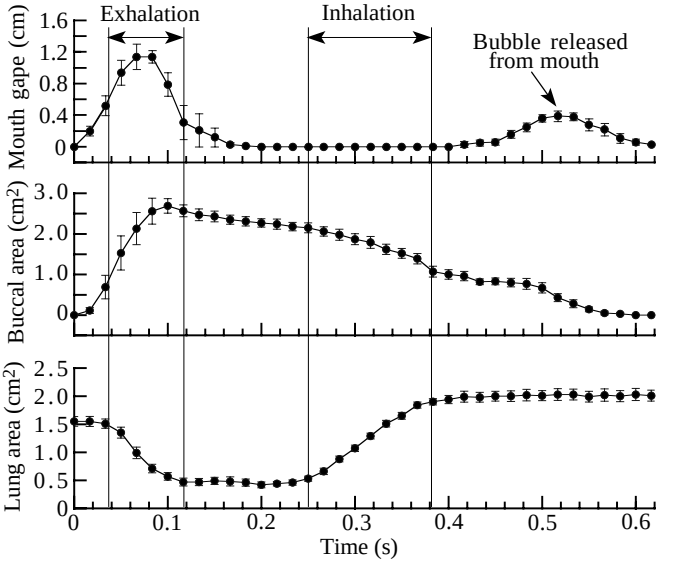

Fig. 2. Lateral-view kinematics of lung ventilation in larval *Ambystoma tigrinum.* Points and error bars represent mean ± S.E.M. from six lateral X-ray sequences from one individual. The upper plot shows the gape of the mouth. The middle plot shows the laterally projected area of air contained in the buccal cavity. The lower plot shows the area of air contained in the lungs, which is equivalent to the area of only one lung because the lungs overlap in lateral projection.

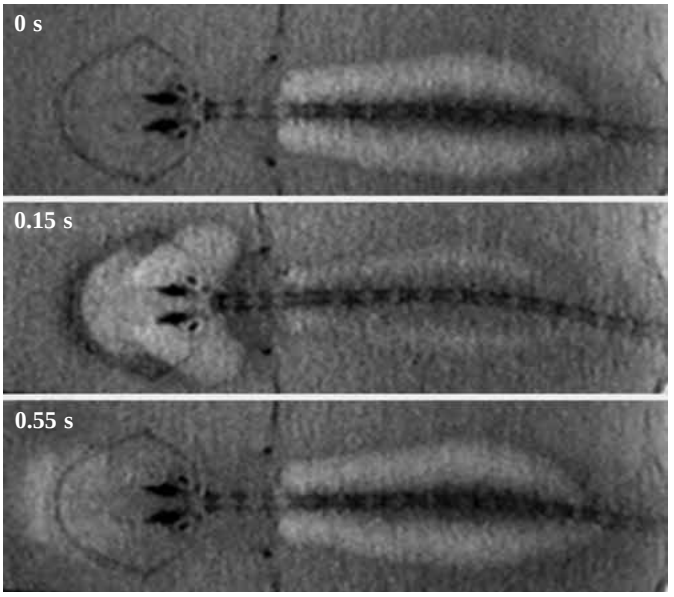

Fig. 3. Still fields from an X-ray video record of lung ventilation in a larval *Ambystoma tigrinum*, dorsoventral view. The fields are printed as X-ray positive images to emphasize the light-colored air in the lungs and buccal cavity. Both lungs are visible in this view. Between 0 and 0.15 s, the buccal cavity fills with air and exhalation occurs. Between 0.15 and 0.55 s, the buccal cavity compresses, filling the lungs with air, and extra air in the buccal cavity exits as bubbles from the mouth.

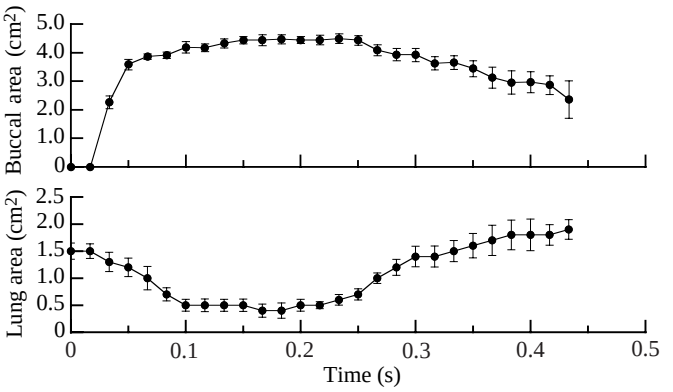

Fig. 4. Dorsoventral-view kinematics of lung ventilation in larval *Ambystoma tigrinum*. Points and error bars represent mean ± S.E.M. from five dorsoventral X-ray sequences from one individual. The upper plot shows the area of air contained in the buccal cavity, and the lower plot shows the area of air contained in one of the two lungs.

Buccal, expired and inspired volumes

Mean areas from lateral and dorsoventral views were combined to provide an estimate of ventilation volumes for each of the three individuals studied (Fig. 5; Table 1). The total volume of air taken into the buccal cavity (V_b) was five times the expired volume from the lungs (V_e) and three times the inspired volume (V_i). The mean value of V_e was 38 % smaller than the mean value of V_i. The mean velocity of buccal expansion for the three individuals was 50.0±2.3 ml s^{-1} (4.0 ml in 0.08 s) and the mean velocity of exhalation from the lungs was 8.9±1.5 ml s^{-1} (0.8 ml in 0.09 s). The mean residual volume in the lungs at the end of exhalation was 0.1±0.06 ml (means ± S.E.M., N=3 individuals).

Buccal and body pressure

Pressures in the buccal and body cavities were recorded synchronously with X-ray videos of air breathing (Fig. 6). The body pressure trace showed a single increase associated with exhalation. The buccal trace was more complex. Buccal pressure dropped below atmospheric pressure as the buccal cavity began to expand (Fig. 6, point 1). It then returned to atmospheric pressure and remained level while the mouth was open. The mouth then closed (point 3), and buccal pressure increased to pump air into the lungs (point 4). The end of the breath was defined as the time at which buccal pressure returned to ambient (point 5), although there was often a subsequent increase in buccal pressure associated with the release of extra air from the buccal cavity.

Six variables were measured from the pressure traces of at least 20 breaths from four individuals (Table 2). In addition, body pressure magnitudes were measured from two individuals in which only body pressure was recorded. The points at which the variables were measured are shown on Fig. 6: P_{body}, the magnitude of body pressure (point 2); P_{buccal}, the magnitude of buccal pressure (point 4); T_{body}, the time from the beginning of mouth opening to peak body pressure (time between points 1 and 2); T_{gape}, the duration of open gape (time between points 1 and 3); T_{buccal}, the time from the beginning of mouth opening to peak buccal pressure (time between points 1 and 4); and T_{total}, the total time required for lung inflation (time between points 1 and 5). One-way ANOVA revealed significant variation between individuals in all variables (Table 2).

Electromyograms of hypaxial musculature

In agreement with the results of Naylor (1978), dissection of four specimens revealed that *A. tigrinum* has four layers of lateral hypaxial musculature: external oblique superficialis (EOS), external oblique profundus (EOP), internal oblique (IO) and transverse abdominis (TA). The EOS is thinner than

Table 1. *Mean ventilation volumes for three larval* Ambystoma tigrinum

	V_b (ml)	V_e (ml)	V_i (ml)	LVE
Larva 01	4.8	1.2	1.8	0.75
Larva 02	3.3	0.7	1.2	0.80
Larva 03	3.7	0.6	1.0	0.85
Mean±S.E.M.	4.0±0.44	0.8±0.20	1.3±0.24	0.80±0.03

Buccal volume (V_b), expired volume (V_e), inspired volume (V_i) and the proportion of fresh air that is pumped into the lung (lung ventilation efficiency, LVE) were calculated from estimated lung and buccal volumes (see Fig. 5).

the other layers, with muscle fibers running from craniodorsal to caudoventral at 54±4.3° to the longitudinal axis of the body. The fibers of the EOP run in the same direction as those of the EOS, but at 16±2.4° to the longitudinal axis of the body. The IO is also essentially longitudinal, with fibers running from cranioventral to caudodorsal at 21±2.9° to the longitudinal axis of the body. The TA is the most transverse of the layers, with fibers running in the same direction as those of the IO, but at a steep angle of 60±2.0° (mean angles ± S.E.M. for N=4 individuals).

Electromyograms (EMGs) were measured from the four layers of lateral hypaxial musculature and the rectus abdominis (RA). EMG activity was observed in all four layers during exhalation in most breaths, and in the RA in approximately half of the breaths. In the four individuals studied, the TA showed EMG signals with the greatest amplitude (Fig. 7). However, the other layers also showed consistent, lower-amplitude activity. To be certain that all the electrodes were working properly, we recorded during undulatory swimming in each animal. These recordings showed that all the electrodes were recording activity, with the exception of the EOS electrode in two animals. Although the TA showed the largest-amplitude activity during exhalation, the EOP tended to show the greatest amplitude during undulation (Fig. 8).

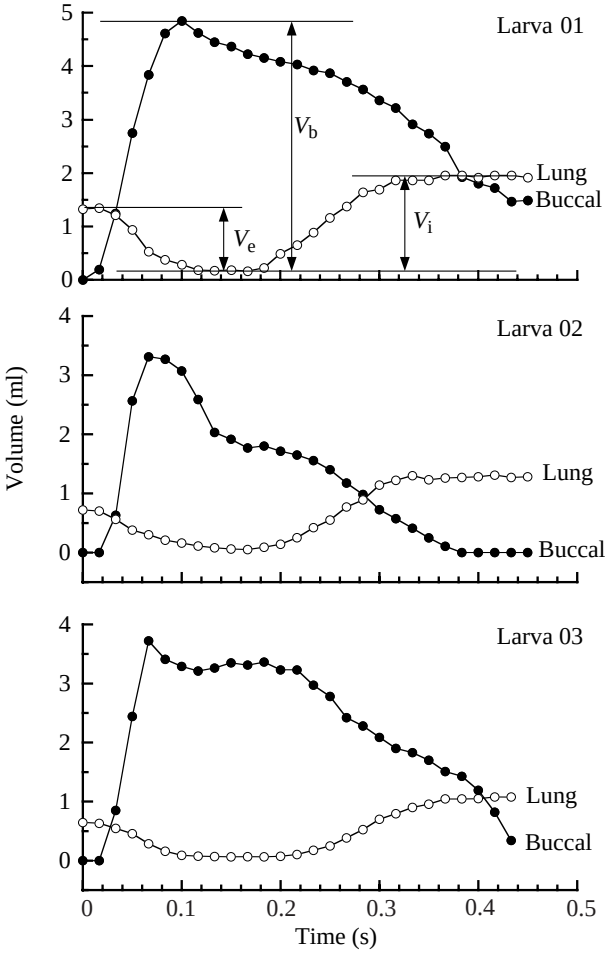

Fig. 5. Buccal and lung volumes estimated by combining results from lateral and dorsoventral X-ray video records for three individuals. For larva 01, six lateral and three dorsoventral breaths were used to calculate the mean volumes; for larva 02 and larva 03, five lateral and five dorsoventral breaths were used. From these plots, maximum buccal volume (V_b), expired volume (V_e) and inspired volume (V_i) can be read. Note the small differences in y-axis scale between individuals.

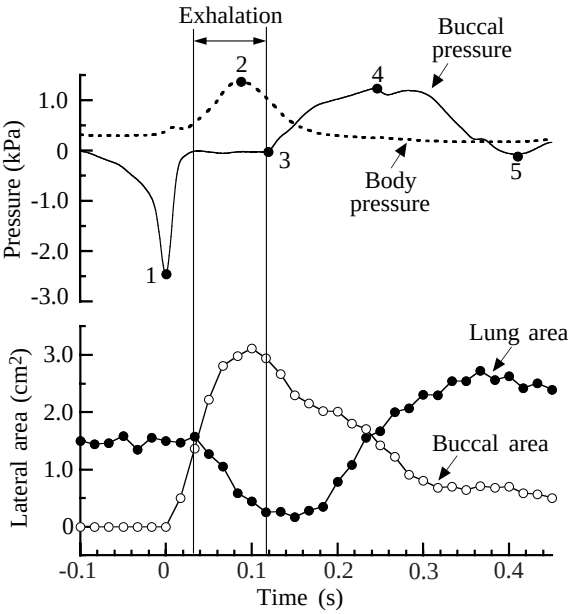

Fig. 6. Buccal and body pressures synchronized with X-ray videos of lung ventilation. The upper panel shows pressure measured in the buccal cavity and in the body cavity outside the lungs. The lower panel shows the projected areas of the lungs and buccal areas in lateral view. For clarity, the pressure waves were filtered with a low-pass filter (20 Hz cutoff) and kinematic plots were smoothed using a three-point running average method. Zero pressure is atmospheric pressure, and the baseline for body pressure is the ambient hydrostatic pressure at the level of the transducer (approximately 3 cm water depth, 0.3 kPa). Points 1–5 refer to variables measured from pressure traces, reported in Table 2.

During exhalation, the TA was active in every breath and showed the earliest onset and longest duration of activity (Fig. 9). The TA became active 10 ms before the body pressure began to rise and ceased activity 15 ms before peak pressure. The EOS, EOP and IO also showed consistent activity, but activity in these muscles began after and ceased before activity in the TA. The RA was the least consistently active, showing activity in only 56 % of breaths (Fig. 9).

One-way ANOVA showed significant differences between individuals in the onset and duration of all the EMG variables. Body pressure magnitudes were also significantly different between individuals. A nested ANOVA indicated that body pressures were significantly larger and more variable when recorded together with EMGs than when recorded alone or with buccal pressure only. When body pressure was measured alone in two individuals with no other instrumentation, mean pressures were 0.47 kPa and 0.62 kPa (Table 2). When body

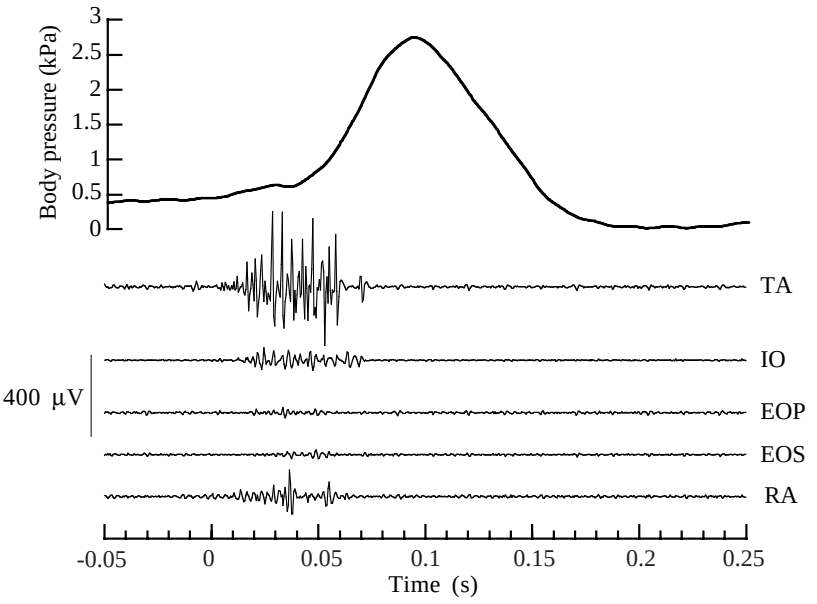

Fig. 7. Electromyograms of the lateral hypaxial musculature recorded simultaneously with pressure in the body cavity during exhalation. Zero pressure is atmospheric pressure, and the baseline for body pressure is the ambient hydrostatic pressure at the level of the transducer (approximately 4 cm water depth, 0.4 kPa). Muscle abbreviations: TA, transverse abdominis; IO, internal oblique; EOP, external oblique profundus; EOS, external oblique superficialis; RA, rectus abdominis.

and buccal pressures were measured simultaneously in four individuals, mean body pressures were 0.39 kPa, with little variation between individuals (Table 2). During EMG recordings, however, larger pressures were recorded. Pressures from one individual were within the range of pressures recorded in the absence of implanted EMG electrodes, (0.53±0.02 kPa), and pressures in another individual were only slightly larger (0.77±0.03 kPa). Body pressure magnitudes were much larger in the two other individuals, with means of 1.25±0.09 and 2.10±0.14 kPa. There were no obvious differences, however, between the EMG signals of individuals for which larger and smaller pressure increases were recorded.

Discussion

The lung ventilation mechanism of larval *Ambystoma tigrinum* is similar in many ways to the breathing mechanisms of previously studied salamanders, *Necturus maculosus* and *Siren lacertina* (Brainerd *et al.* 1993; Brainerd and Monroy, 1998). All three salamanders use a two-stroke buccal pump for inspiration, and all use the transverse abdominis (TA) muscle to power active exhalation. However, larval *A. tigrinum* differs in that all of its lateral hypaxial muscle layers are active during exhalation, not just the TA as in *N. maculosus* and *S. lacertina*.

Two-stroke buccal pumping and ventilation efficiency

X-ray video records revealed that larval tiger salamanders use a two-stroke buccal pump for lung ventilation: exhalation and inhalation occur within one buccal expansion–compression cycle, and expired air mixes with fresh air in the buccal cavity (Figs 1–5). A breath begins as the mouth opens, and the buccal cavity expands at a mean velocity of 50 ml s^{-1}. During this rapid buccal expansion, expired gas flows from the lungs into the buccal cavity at a considerably slower velocity, 8.9 ml s^{-1}. Thus, the air flowing into the buccal cavity comes

Table 2. *Variation between individuals of larval* Ambystoma tigrinum *in timing and magnitude of buccal and body pressure*

	N	P_{body}* (kPa)	P_{buccal}*** (kPa)	T_{body}** (ms)	T_{gape}*** (ms)	T_{buccal}*** (ms)	T_{total}*** (ms)
Larva A	21	0.47±0.05	–	–	–	–	–
Larva B	20	0.62±0.07	–	–	–	–	–
Larva C	22	0.39±0.05	1.84±0.10	95±5	160±5	404±6	561±8
Larva D	20	0.40±0.07	1.25±0.12	107±5	158±4	366±6	469±6
Larva E	20	0.41±0.03	3.80±0.38	88±3	142±5	372±7	514±15
Larva F	20	0.36±0.05	1.03±0.07	96±5	100±6	354±18	501±26
Mean ± s.e.m.		0.44±0.04 (*N*=6)	1.98±0.63 (*N*=4)	97±4 (*N*=4)	140±14 (*N*=4)	374±11 (*N*=4)	511±20 (*N*=4)

Means ± S.E.M. are given for each individual.
Body pressure only was measured in two individuals, and body and buccal pressure in four individuals.
Significant between-individual variation is indicated (*P<0.05; **P<0.01; ***P<0.001).
Variables are defined in the text (see also Fig. 6).

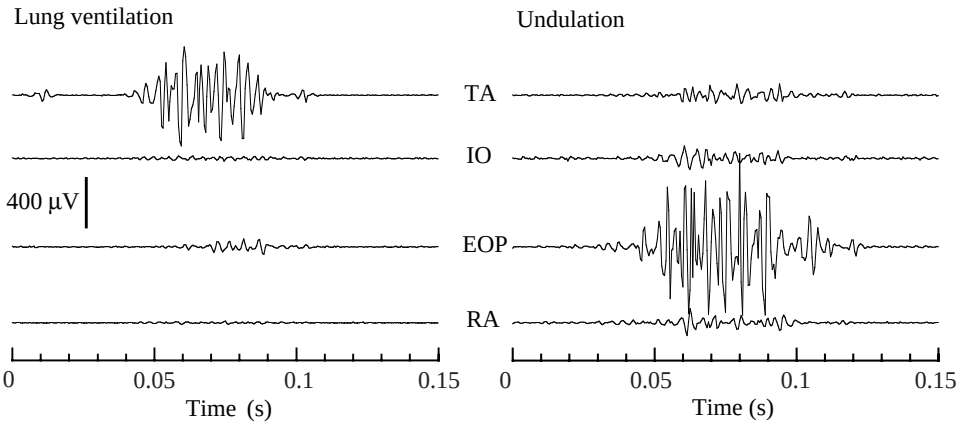

Fig. 8. Electromyograms of the lateral hypaxial musculature during lung ventilation and swimming by lateral undulation. The external oblique superficialis is not shown because this electrode did not record any muscle activity in this individual. Muscle abbreviations: TA, transverse abdominis; IO, internal oblique; EOP, external oblique profundus; RA, rectus abdominis.

from two sources: fresh air flows into the mouth, and expired air flows out from the lungs. Given that the buccal cavity expands more than five times faster than air flows out of the lungs, fresh air must be flowing into the mouth at a high velocity (50–8.9=41.1 ml s^{-1}), and the expired air cannot leave the mouth against this inflow. The expired air mixes with fresh air in the buccal cavity, and some of this mixture (30 %) is pumped into the lungs. The remaining air in the buccal cavity is then expelled through the gill slits and mouth (Figs 1–3).

Because expired air is pumped back into the lungs, this two-stroke breathing mechanism might be expected to result in a rather poor lung ventilation efficiency (LVE, defined as the percentage of fresh air that is inspired). However, the small volume of expired air (0.8 ml) in the buccal cavity is diluted by a fourfold larger volume of fresh air (3.2 ml), such that the final mixture is 80 % fresh air (LVE=3.2/4.0). In salamanders, there is no anatomical dead space (since the lungs originate close to the glottis), but the expired air retained in the buccal cavity can be viewed as analogous to the dead space in mammalian lungs. For comparison, in a 70 kg human breathing at rest, the tidal volume is 500 ml and the dead space is 150 ml, indicating that 350 ml of fresh air is mixing with the expired air in the dead space, yielding an LVE of 70 % (Guyton and Hall, 1997). Thus, the two-stroke pump is comparable in efficiency with the mammalian aspiration system, although it is less efficient than a four-stroke buccal pump, in which no expired air is rebreathed (100 % LVE; Brainerd *et al.* 1993).

Larval tiger salamanders exhale almost completely and exchange a large proportion of the air in their lungs with each breath (Figs 1, 5). The mean residual volume in the lungs at the end of exhalation is 0.1 ml, which mixes with 1.3 ml of inspired air, of which 1.0 ml is fresh air and 0.3 ml is expired air. As a result, the final lung volume is 1.4 ml, of which 71 % is fresh air. Other aquatic salamanders studied to date also exhale most of the air from their lungs with each breath (Martin and Hutchison, 1979; Brainerd *et al.* 1993; Brainerd and Monroy, 1998), but terrestrial salamanders have much smaller tidal volumes relative to their total lung volumes (E. L. Brainerd, personal observation).

The mean expired volumes in larval tiger salamanders were 38 % smaller than the inspired volumes (Table 1; Fig. 5). Part of this difference is probably due to oxygen removed from the air, which is not replaced by an equal volume of CO_2 because most of the CO_2 is exchanged through the skin and gills (Whitford and Sherman, 1968; Heath, 1976; Boutilier, 1984). However, even if all the oxygen were extracted and no CO_2 replaced, oxygen uptake could account for a maximum difference of only 21 % between the expiratory and inspiratory volumes. The mean difference is greater than 21 %, a finding

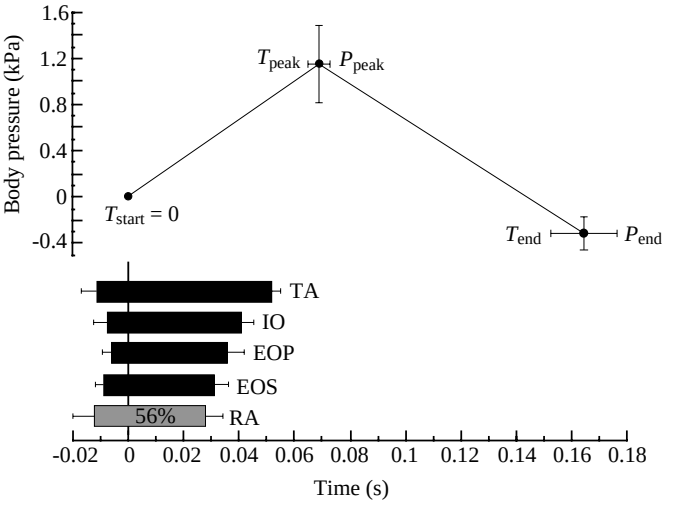

Fig. 9. Summary diagram of body pressures and hypaxial muscle electromyograms (EMGs) from at least 20 breaths from each of four individual larvae. Means of four body pressure variables and two electromyogram variables per muscle are shown ± S.E.M. Means are calculated from the means for each individual, with standard errors calculated using N=4 (not the total number of breaths). For EOS, N=2 because this electrode did not work in two individuals. Zero time (T_{start}) is defined as the time that body pressure began to rise, and zero pressure is the hydrostatic pressure at the level of the transducer. In the upper panel, mean values for peak body pressure (P_{peak}), the time to peak pressure (T_{peak}), the time when the pressure wave ends (T_{end}) and the pressure at the end of the wave (P_{end}) are shown. In the lower panel, the onset time for each muscle relative to T_{start} and the duration of EMG activity are shown. The RA was active in only 56 % of breaths. Muscle abbreviations: TA, transverse abdominis; IO, internal oblique; EOP, external oblique profundus; EOS, external oblique superficialis; RA, rectus abdominis.

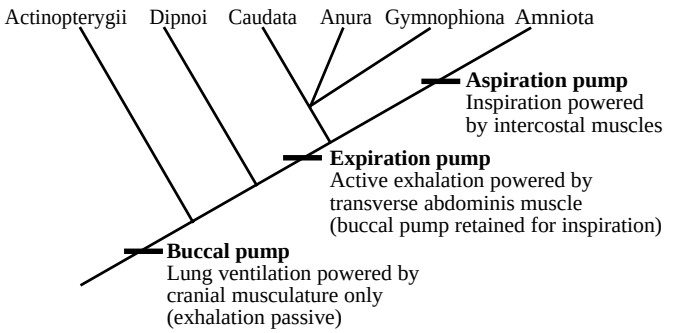

Fig. 10. Simplified phylogeny of air-breathing vertebrates with a hypothesis for the evolution of aspiration breathing from buccal pump breathing (Brainerd *et al.* 1993). Ray-finned fishes (Actinopterygii) and lungfishes (Dipnoi) utilize a buccal pump for lung ventilation. Both salamanders (Caudata) and amniotes use the transverse abdominis for exhalation (expiration pump). Amniotes inhale using an aspiration pump.

that can be explained by the observation that the animals sometimes exhaled small bubbles of air while submerged between breaths.

The two-stroke buccal pump of larval *A. tigrinum* is almost identical to the breathing mechanism of *Necturus maculosus* (Brainerd *et al.* 1993). Both exhale while the buccal cavity is expanding rapidly, causing all the expired air to be retained in the buccal cavity. The only mechanism available to increase the LVE is to increase the total volume of air taken into the buccal cavity, thus diluting the expired air with as much fresh air as possible. In contrast, other two-stroke breathers have developed more complex breathing patterns that may serve to increase the LVE. The simplest of these is the addition of accessory inspirations after the first two-stroke, expiratory–inspiratory cycle. The first cycle of buccal expansion and compression pumps mixed air into the lungs, but no expiration occurs on subsequent cycles, and fresh unmixed air is pumped into the lungs. The use of one and sometimes two accessory inspirations has been observed in a lungfish, *Lepidosiren paradoxa* (Bishop and Foxon, 1968; Brainerd, 1994), and in an aquatic salamander, *Siren lacertina* (Brainerd and Monroy, 1998). In caecilians, such as *Dermophis mexicanus*, this mechanism is highly developed, with a mean of 16 fresh-air inspirations after the first expiratory–inspiratory cycle (Carrier and Wake, 1995).

Another strategy for increasing LVE is seen in the lungfishes *Protopterus aethiopicus* and *Lepidosiren paradoxa* and in a salamander, *Siren lacertina*. These animals begin a breath by expanding the buccal cavity only partially, and then stop expanding the cavity during exhalation. When exhalation has been completed, the buccal cavity expands more fully, thus filling with mostly fresh air (Brainerd, 1994; Brainerd and Monroy, 1998).

Frogs increase their LVE by performing a series of asymmetrical, two-stroke breaths that lead to a net inflation or deflation of the lungs (e.g. de Jongh and Gans, 1969; Wang, 1994). During an inflation series, the volume of air exhaled in each breath is reduced and thus the proportion of air that is rebreathed is reduced. In addition, it has been suggested that bullfrogs (*Rana catesbeiana*) use a jet-stream effect to cause expired air to flow through the buccal cavity without mixing substantially with the fresh air already contained there (de Jongh and Gans, 1969; Gans *et al.* 1969). When the jet-stream hypothesis was tested using blowhole pneumotachography in *Rana pipiens* (Vitalis and Shelton, 1990), little evidence for a coherent jet stream of expired air from the lungs was found. A jet stream is highly unlikely in larval tiger salamanders because air is flowing rapidly into the mouth during exhalation.

Active and passive exhalation in salamanders

Measurements of body cavity pressure synchronized with X-ray video recordings demonstrate that larval *A. tigrinum* exhale actively (Fig. 6). Body cavity pressure begins to increase shortly before exhalation begins, and exhalation ends as body pressure begins to decline. Exhalation is very rapid, occupying only approximately 0.1 s, and the increase in mean body pressure is 0.44 kPa (Table 2). Hypaxial muscle activity begins shortly before body pressure starts to increase and ceases shortly before pressure begins to decline (Figs 7, 9).

Previous results have shown that exhalation is also active and fast in another aquatic salamander, *Necturus maculosus* (Brainerd *et al.* 1993). Exhalation occurs in approximately 0.1 s in *N. maculosus*, and the mean increase in body pressure is 0.70 kPa. Exhalation is much slower and partially passive in two elongate, aquatic salamanders, *Siren lacertina* and *Amphiuma tridactylum* (Brainerd and Dumka, 1995; Brainerd and Monroy, 1998). Exhalation takes approximately 0.5 s in *S. lacertina* and more than 2 s in *Amphiuma tridactylum*. The first part of exhalation is passive in these salamanders, and body pressure then increases in the second half of exhalation to force the remaining air out of the lungs. The magnitude of the increase in body pressure is smaller in *S. lacertina* than in larval *A. tigrinum* and *N. maculosus* (mean increase 0.20 kPa; Brainerd and Monroy, 1998).

It is unclear why two distantly related, aquatic, non-elongate salamanders (*A. tigrinum* and *N. maculosus*) exhale rapidly, whereas two distantly related, aquatic, elongate salamanders (*S. lacertina* and *Amphiuma tridactylum*) exhale more slowly. An advantage of slower exhalation could be that a significant proportion of the slow exhalation can be powered by passive mechanisms, thus requiring less energy than fast exhalation. Hydrostatic pressure is an important component of the passive phase of exhalation in elongate salamanders (Brainerd and Dumka, 1995; Brainerd and Monroy, 1998). During exhalation, elongate salamanders experience a larger gradient in hydrostatic pressure than do non-elongate salamanders because the caudal ends of their lungs are deeper in the water. Thus, elongate salamanders may breathe more slowly to take advantage of hydrostatic pressure to power the first half of expiration.

Electromyographic (EMG) recordings of the hypaxial muscle layers in larval *A. tigrinum* indicate that all four layers of lateral hypaxial musculature are active during exhalation,

and the rectus abdominis is sometimes active (Figs 7–9). The transverse abdominis (TA) showed the longest-duration, highest-amplitude and most consistent activity of the four layers, suggesting that the TA is the primary expiratory muscle in larval *A. tigrinum*.

These results differ from previous results of EMG recordings during exhalation in two other salamander species, *Necturus maculosus* and *Siren lacertina* (Brainerd *et al.* 1993; Brainerd and Monroy, 1998). In *N. maculosus* and *S. lacertina*, only the TA is active during exhalation, whereas in larval *A. tigrinum*, the external oblique superficialis (EOS), external oblique profundus (EOP) and internal oblique (IO) are also active. It is somewhat surprising to see activity in the EOP and IO, since these layers have quite low muscle fiber angles (16 ° and 21 ° to the horizontal axis, respectively) and thus have relatively poor mechanical advantage to compress the body cavity for exhalation. The TA has the most-transverse fibers of any of the layers; it therefore has the best mechanical advantage for producing exhalation.

All four layers of lateral hypaxial musculature in larval *A. tigrinum*, including the TA, are extremely thin. Even though the other layers have poor mechanical advantage, they may contribute to exhalation in larval tiger salamanders because the TA is so thin. It is also possible that the discomfort of EMG electrodes caused greater activity in the hypaxial layers than might normally be present when the animals are breathing quietly at rest. Two of the four individuals generated body pressures that were 2–6 times larger than the mean pressures recorded in animals without EMG electrodes (compare Figs 6 and 7). However, two of the individuals generated body pressures that were similar to non-EMG pressures, and all four layers were active in these individuals as well.

The evolution of aspiration breathing

The finding that the TA muscle is active during exhalation in larval tiger salamanders contributes to a growing body of evidence that the use of the TA for exhalation is a primitive character for salamanders (Brainerd *et al.* 1993; Brainerd and Monroy, 1998). Members of three families of salamanders, Sirenidae, Proteidae and Ambystomatidae, have now been shown to use the TA for exhalation. In all groups of amniotes, the TA also contributes to active exhalation, which is remarkable in view of the highly diverse body forms of amniotes (turtles, Gaunt and Gans, 1969; snakes, Rosenberg, 1973; crocodilians, Gans and Clark, 1976; mammals, De Troyer and Loring, 1986; birds, Fedde, 1987; lizards, Carrier, 1989). This finding, combined with evidence that the use of the TA for exhalation is primitive for salamanders, suggests that the use of the TA to power an active ‘expiration pump’ is a primitive character for tetrapods (Fig. 10). This conclusion is supported by the observation that the TA muscle is present only in tetrapods and is absent from lungfishes, which exhale passively.

Frogs (Anura) and caecilians (Gymnophiona) have highly derived body forms; their exhalation mechanisms therefore yield little reliable information about the evolution of TA function. In frogs, the lateral hypaxial musculature is reduced from four layers to two, and these two layers are usually called EO and IO, although homology is uncertain. Exhalation during quiet breathing in frogs is primarily produced by elastic recoil of the lungs (de Jongh and Gans, 1969), but hypaxial muscles produce forceful, active expirations during vocalization (Martin and Gans, 1972). Caecilians exhale passively, a behavior that appears to be a derived condition related to the high internal pressures that they generate for locomotion (Carrier and Wake, 1995; O’Reilly *et al.* 1997; Summers and O’Reilly, 1997).

In the light of the finding that salamanders and amniotes share the use of the TA for exhalation, and since salamanders retain the most primitive body form of the extant amphibians, it is reasonable to conclude that an expiration pump powered by the TA is a synapomorphy for tetrapods (Fig. 10). If this is so, it appears likely that aspiration breathing evolved from buccal pumping in two stages: first, from a buccal pump to an expiration pump in which body muscles are recruited for exhalation (as seen in salamanders); and second, to an aspiration pump in which body muscles are used for both exhalation and inhalation (as seen in amniotes).

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References

BISHOP, I. R. AND FOXON, G. E. H. (1968). The mechanism of breathing in the South American lungfish, *Lepidosiren paradoxa*; a radiological study. *J. Zool., Lond.* **154**, 263–271.

BOUTILIER, R. G. (1984). Characterization of the intermittent breathing pattern in *Xenopus laevis*. *J. exp. Biol.* **110**, 291–309.

BOUTILIER, R. G., MCDONALD, D. G. AND TOEWS, D. P. (1980). The effects of enforced activity on ventilation, circulation and blood acid–base balance in the aquatic gill-less urodele, *Cryptobranchus alleganiensis*: a comparison with the semi-terrestrial *Bufo marinus*. *J. exp. Biol.* **84**, 289–302.

BRAINERD, E. L. (1994). The evolution of lung–gill bimodal breathing and the homology of vertebrate respiratory pumps. *Am. Zool.* **34**, 289–299.

BRAINERD, E. L., DITELBERG, J. S. AND BRAMBLE, D. M. (1993). Lung ventilation in salamanders and the evolution of vertebrate air-breathing mechanisms. *Biol. J. Linn. Soc.* **49**, 163–183.

BRAINERD, E. L. AND DUMKA, A. M. (1995). Mechanics of lung ventilation in an aquatic salamander, *Amphiuma tridactylum*. *Am. Zool.* **35**, 20A.

BRAINERD, E. L. AND MONROY, J. A. (1998). Mechanics of lung ventilation in a large aquatic salamander, *Siren lacertina*. *J. exp. Biol.* **201**, 673–682.

BRETT, S. S. AND SHELTON, G. (1979). Ventilatory mechanisms of the amphibian *Xenopus laevis;* the role of the buccal force pump. *J. exp. Biol.* **80**, 251–269.

BURGGREN, W. W. AND WOOD, S. C. (1981). Respiration and acid–base balance in the tiger salamander, *Ambystoma tigrinum*: influence of temperature acclimation and metamorphosis. *J. comp. Physiol.* **144**, 241–246.

CARRIER, D. R. (1989). Ventilatory action of the hypaxial muscles of the lizard *Iguana iguana:* a function of slow muscle. *J. exp. Biol.* **143**, 435–457.

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.* **180**, 75–83.

CARRIER, D. R. AND WAKE, M. H. (1995). Mechanism of lung ventilation in the caecilian *Dermophis mexicanus*. *J. Morph.* **226**, 289–295.

DE JONGH, H. J. AND GANS, C. (1969). On the mechanism of respiration in the bullfrog *Rana catesbeiana*: a reassessment. *J. Morph.* **127**, 259–290.

DE TROYER, A. AND LORING, S. H. (1986). Action of the respiratory muscles. In *Handbook of Physiology*, section 3, *The Respiratory System*, vol. III, *Mechanics of Breathing*, part 2 (ed. A. P. Fishman, P. T. Mackelm, J. Mead and S. R. Geiger), pp. 443–461. Bethesda, MD: American Physiological Society.

DEYST, K. A. AND LIEM, K. F. (1985). The muscular basis of aerial ventilation of the primitive lung of *Amia calva*. *Respir. Physiol.* **59**, 213–223.

FEDDE, M. R. (1987). Respiratory muscles. In *Bird Respiration*, vol. I (ed. T. J. Seller), pp. 3–37. Boca Raton, FL: CRC Press.

GANS, C. (1970). Strategy and sequence in the evolution of the external gas exchangers of ectothermal vertebrates. *Forma et Functio* **3**, 61–104.

GANS, C. AND CLARK, B. (1976). Studies on the ventilation of *Caiman crocodilus* (Crocodilia, Reptilia). *Respir. Physiol.* **26**, 285–301.

GANS, C., DE JONGH, H. J. AND FARBER, J. (1969). Bullfrog (*Rana catesbeiana*) ventilation: how does the frog breathe? *Science* **163**, 1223–1225.

GAUNT, A. S. AND GANS, C. (1969). Mechanics of respiration in the snapping turtle, *Chelydra serpentina* (Linne). *J. Morph.* **128**, 195–228.

GUIMOND, R. W. AND HUTCHISON, V. H. (1976). Gas exchange of the giant salamanders of North America. In *Respiration of Amphibious Vertebrates* (ed. G. M. Hughes), pp. 313–338. New York: Academic Press.

GUYTON, A. C. AND HALL, J. E. (1997). *Human Physiology and Mechanisms of Disease*. Philadelphia: W. B. Saunders. 737pp.

HEATH, A. G. (1976). Respiratory responses to hypoxia by *Ambystoma tigrinum* larvae, paedomorphs and metamorphosed adults. *Comp. Biochem. Physiol.* **55**A, 45–49.

JAYNE, B. C., LAUDER, G. V., REILLY, S. M. AND WAINWRIGHT, P. C. (1990). The effect of sampling rate on the analysis of digital electromyograms from vertebrate muscle. *J. exp. Biol.* **154**, 557–565.

LIEM, K. F. (1985). Ventilation. In *Functional Vertebrate Morphology* (ed. M. Hildebrand, D. M. Bramble, K. F. Liem and D. B. Wake), pp. 185–209. Cambridge, MA: Harvard University Press.

LOEB, G. E. AND GANS, C. (1986). *Electromyography for Experimentalists*. Chicago: University of Chicago Press. 373pp.

MARTIN, K. M. AND HUTCHISON, V. H. (1979). Ventilatory activity in *Amphiuma tridactylum* and *Siren lacertina* (Amphibia, Caudata). *J. Herpetol.* **13**, 427–434.

MARTIN, W. F. AND GANS, C. (1972). Muscular control of the vocal tract during release signaling in the toad, *Bufo valliceps*. *J. Morph.* **137**, 1–28.

MCMAHON, B. R. (1969). A functional analysis of aquatic and aerial respiratory movements of an African lungfish, *Protopterus aethiopicus*, with reference to the evolution of the lung-ventilation mechanism in vertebrates. *J. exp. Biol.* **51**, 407–430.

MOON, B. R. (1996). Sampling rates, aliasing and the analysis of electrophysiological signals. *IEEE Proc. 15th Southern Biomed. Engin. Conf.* **1996**, 401–404.

NAYLOR, B. G. (1978). The systematics of fossil and recent salamanders with special reference to the vertebral column and trunk musculature. PhD thesis, University of Alberta, Edmonton, Alberta. 641pp.

O'REILLY, J. C., RITTER, D. A. AND CARRIER, D. R. (1997). Hydrostatic locomotion in a limbless tetrapod. *Nature* **386**, 269–272.

ROSENBERG, H. I. (1973). Functional anatomy of pulmonary ventilation in the garter snake, *Thamnophis elegans*. *J. Morph.* **140**, 171–184.

SUMMERS, A. P. AND O'REILLY, J. C. (1997). A comparative study of locomotion in the caecilians *Dermophis mexicanus* and *Typhlonectes natans* (Amphibia: Gymnophiona). *Zool. J. Linn. Soc.* **121**, 65–76.

TOEWS, D. (1973). Oxygen consumption in *Amphiuma tridactylum*. *Can. J. Zool.* **51**, 664–666.

ULTSCH, G. R. AND DUKE, J. T. (1990). Metabolic oxygen regulation and conformity during submergence in the salamanders *Siren lacertina*, *Amphiuma means* and *Amphiuma tridactylum*. *Oecologia* **84**, 16–23.

VITALIS, T. Z. AND SHELTON, G. (1990). Breathing in *Rana pipiens*: the mechanism of ventilation. *J. exp. Biol.* **154**, 537–556.

WANG, T. (1994). Measurement of ventilatory responses in the toad *Bufo marinus*: a comparison of pneumotachography and buccal pressures. *Comp. Physiol. Biochem.* **109**A, 793–798.

WHITFORD, W. AND SHERMAN, R. (1968). Aerial and aquatic respiration in axolotl and transformed *Ambystoma tigrinum*. *Herpetologica* **24**, 233–237.