MECHANICS OF LUNG VENTILATION IN A LARGE AQUATIC SALAMANDER, *SIREN LACERTINA*

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Summary

Lung ventilation in *Siren lacertina* was studied using X-ray video, measurements of body cavity pressure and electromyography of hypaxial muscles. *S. lacertina* utilizes a two-stroke buccal pump in which mixing of expired and inspired gas is minimized by partial expansion of the buccal cavity during exhalation and then full expansion after exhalation is complete. Mixing is further reduced by the use of one or two accessory inspirations after the first, mixed-gas cycle. Exhalation occurs in two phases: a passive phase in which hydrostatic pressure and possibly lung elasticity force air out of the lungs, and an active phase in which contraction of the transverse abdominis (TA) muscle increases body cavity pressure and forces most of the remaining air out. In electromyograms of the lateral hypaxial musculature, the TA became active 200–400 ms before the rise in body cavity pressure, and activity ceased at peak pressure. The TA was not active during inspiration, and no consistent activity during breathing was noted in the external oblique, internal oblique and rectus abdominis muscles. The finding that the TA is the primary expiratory muscle in *S. lacertina* agrees with findings in a previous study of another salamander, *Necturus maculosus*. Together, these results indicate that the use of the TA for exhalation is a primitive character for salamanders and support the hypothesis that the breathing mechanism of salamanders represents an intermediate step in evolution between a buccal pump, in which only head muscles are used for ventilation, and an aspiration pump, in which axial muscles are used for both exhalation and inhalation.

Key words: respiration, air-breathing, aspiration, buccal pump, pulse pump, exhalation, evolution, physiology, Amphibia, Lissamphibia, Urodela, hypaxial muscles, salamander, *Siren lacertina*.

Introduction

Lung ventilation in vertebrates is accomplished by diverse respiratory pump mechanisms. Amniotes use an aspiration pump in which the muscles of the thorax and abdomen expand the body and pull air into the lungs. Air-breathing fishes and amphibians, in contrast, use a buccal pump (pulse pump) in which the mouth cavity expands to fill with fresh air and then compresses to pump air into the lungs (Gans, 1970*b*; Liem, 1985). The phylogenetic distribution of breathing mechanisms indicates that the buccal pump is a primitive feature of Osteichthyes and the aspiration pump is a derived feature of Amniota (Fig. 1).

Vertebrate biologists have long puzzled over the evolutionary transition from buccal pumping to aspiration breathing (e.g. Noble, 1931). Previous studies of this transition have been aimed at determining whether the earliest tetrapods were aspiration breathers or buccal pumpers (Gans, 1970*b*; Romer, 1972; Packard, 1976). Emphasis has centered on the structure of the ribs in amphibian fossils and on the presumed physiological requirements of terrestrial *versus* aquatic life. It has been difficult to draw firm conclusions from these studies, however, because of the inherent uncertainties of assigning physiological functions to extinct animals.

It has generally been concluded that the small, smooth-skinned modern amphibians are so different from the large, armored early tetrapods that studies of living amphibians will yield little information on the respiratory mechanics of extinct tetrapods (Gans, 1970*a*; Romer, 1972; Packard, 1976). This conclusion is probably true for frogs, given that they have a highly derived body form and modifications of the respiratory tract for vocalization (Gans, 1970*a*). This conclusion is also likely to be true for caecilians, which have modified their body plan for fossorial life. Salamanders, however, have retained a more primitive body form, and a recent study of lung ventilation in *Necturus maculosus* has suggested that studies of salamanders may indeed yield useful information on the evolution of aspiration breathing (Brainerd *et al.* 1993).

The mechanism of lung ventilation in *N. maculosus* is similar to the buccal pump used by lepidosirenid lungfishes (Bishop and Foxon, 1968; McMahon, 1969; Brainerd *et al.* 1993; Brainerd, 1994). *N. maculosus*, however, differs from lungfishes by exhaling actively, contracting its hypaxial

Fig. 1. 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 pure buccal pump, in which no axial musculature is used for lung ventilation. Amniotes use an aspiration pump, in which both exhalation and inhalation are powered by axial musculature. On the basis of a study of one urodele species, *Necturus maculosus*, Brainerd *et al.* (1993) proposed that the breathing mechanism of salamanders is intermediate between buccal pumping and aspiration breathing: a buccal pump is used for inspiration, but exhalation is active, powered by contraction of the transverse abdominis

(TA) muscle (expiration pump). Amniotes also use the TA for active exhalation, and thus it is proposed that the expiration pump is primitive for tetrapods. The present study tests this hypothesis by determining whether *Siren lacertina*, a member of the basal urodele family Sirenidae, also utilizes an expiration pump.
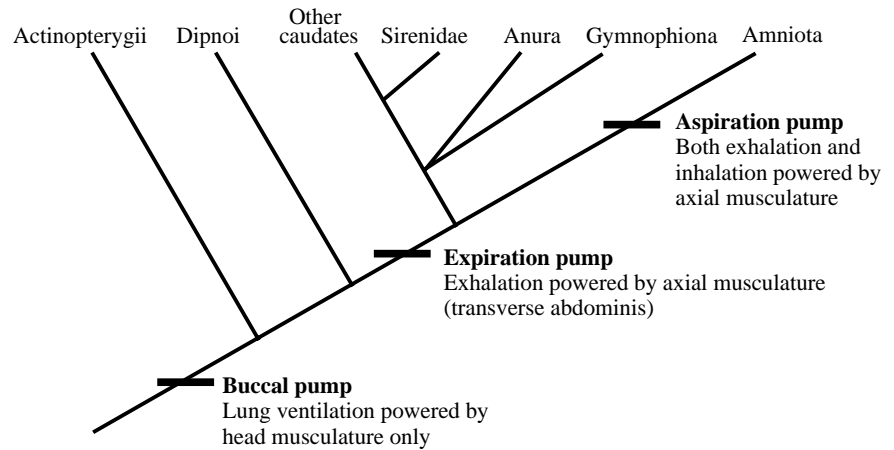
musculature to increase the abdominal pressure and to force air out of the lungs. In contrast, lungfishes exhale passively by hydrostatic pressure and elastic recoil of the lungs and body wall. Electromyograms of the four layers of lateral hypaxial musculature in *N. maculosus* indicate that the transverse abdominis is the primary muscle powering exhalation, with a possible secondary contribution from the internal oblique (Brainerd *et al.* 1993).

The finding that *N. maculosus* uses hypaxial muscles for exhalation is remarkable because it is the first documentation of axial musculature being used for breathing in any non-amniote. This mechanism for exhalation is called an 'expiration pump', in contrast with the aspiration pump of amniotes in which the axial musculature is used for both exhalation and inhalation. The primitive breathing mechanism of amniotes is thought to have resembled that of *Iguana iguana*, in which the transverse abdominis and retrahentes costarum power active exhalation, and the intercostal muscles rotate the ribs and expand the thorax for inhalation (Carrier, 1989). *N. maculosus* also uses the transverse abdominis for exhalation, but inspires by pumping air from the oral cavity into the lungs. The results from *N. maculosus* suggest that aspiration breathing may have evolved in two steps (Fig. 1): first, from no involvement of axial musculature in breathing to the use of axial muscles for exhalation and a buccal pump for inhalation (as in *N. maculosus*) and, second, to the use of axial muscles for both exhalation and inhalation (Brainerd *et al.* 1993).

The foregoing hypothesis of evolutionary transitions is based, however, on results from just one salamander species. It is possible that *N. maculosus* is unusual in its use of axial muscles for exhalation. *N. maculosus* normally lives in deep, well-oxygenated water and relies primarily on its skin and external gills for gas exchange (Guimond and Hutchison, 1972, 1976). The lungs in *N. maculosus* are thought to serve primarily as buoyancy organs, and lung ventilation may occur infrequently in nature. Thus, it is possible that the lung

ventilation mechanism of *N. maculosus* is derived, and the purpose of the present study is to investigate the mechanics of respiration in another salamander, *Siren lacertina*, in order to determine whether active exhalation is primitive for salamanders.

S. lacertina (the greater siren) was chosen for this study for two reasons: (1) in its natural environment, *S. lacertina* is more dependent on the lungs for gas exchange than most other salamanders; and (2) the family Sirenidae, to which *S. lacertina* belongs, is the sister group to all other caudates (Fig. 1).

S. lacertina is fully aquatic and lives in swamps and drainage ditches in the southeastern United States. It is paedomorphic, with external gills, an elongate body, a well-developed median fin-fold and lateral-line system, reduced forelimbs and no pelvic girdle or hindlimbs. *S. lacertina* is capable of trimodal gas exchange through the skin, gills and lungs. In adults, the gills are small relative to body size and do not play a major role in oxygen uptake, but they are important in the release of carbon dioxide into the water (Guimond and Hutchison, 1973, 1976). The lungs of *S. lacertina*, however, are well vascularized and highly septate, and thus appear well designed for gas exchange (based on Guimond and Hutchison, 1973, and studies of *S. intermedia* by Czopek, 1965).

In cool (<20 °C) well-oxygenated water, *S. lacertina* relies primarily on the skin for oxygen uptake (Guimond and Hutchison, 1973; Shield and Bentley, 1973; Ultsch, 1974; Duke and Ultsch, 1990). In warmer, less well-oxygenated water, however, the lungs become more important for gas exchange. At 25 °C, the lungs are responsible for up to 75 % of oxygen uptake and 60 % of carbon dioxide elimination (Guimond and Hutchison, 1973). The natural environment of *S. lacertina* is very warm in the summer months, and the ponds, swamps and ditches are often hypoxic and occasionally anoxic (Ultsch, 1976). Thus, during part of the year, *S. lacertina* is an obligate air-breather.

S. lacertina is a member of the family Sirenidae, which most hypotheses of urodele phylogeny place as the sister group of all other salamanders (Fig. 1; Duellman and Trueb, 1986; Larson and Dimmick, 1993). Because the purpose of this study is to determine whether the use of an expiration pump is primitive for salamanders, the phylogenetic position of sirenids makes *S. lacertina* a good choice for this study. If we find that *S. lacertina* uses an expiration pump, then we shall conclude that the use of axial muscles for exhalation is a primitive condition for salamanders. If *S. lacertina* does not use an expiration pump, then further work will be required to determine whether active exhalation is a derived condition found only in some salamanders or a primitive condition that has been lost in sirenids.

Materials and methods

Specimens

Five *Siren lacertina* L. (26.5–38.0 cm snout–vent length, SVL) were obtained in late spring (April–May) from a scientific supplier (Sullivan's Amphibians, Nashville, TN, USA). *S. lacertina* is a fully aquatic salamander, and animals were therefore kept individually in water-filled, 76 l aquaria at room temperature (21–23 °C). Recordings of breathing were made during the day. Animals were fed twice a week on a diet of large earthworms.

For surgical implantation of pressure cannulae and electrodes, animals were anesthetized in a 1 g l^{-1} solution of tricaine methanesulfonate (Finquel, Argent). The animals were carefully monitored for approximately 20 min as the anesthetic took effect. Upon revival, the animals were again watched closely to ensure a safe recovery.

All recordings were made with the animals in air-equilibrated water at room temperature (21–23 °C). We found that the animals took air-breaths regularly (at least 5 breaths h^{-1}) in water at room temperature. Therefore, we did not find it necessary to reduce aerial or aquatic oxygen levels to induce air-breathing behavior.

X-ray videography

X-ray videos of *S. lacertina* were taken at a rate of 60 fields s^{-1} by means of a Siemens X-ray cine apparatus and a Sony DCR VX1000 digital camcorder (shutter speed, 1/250 s). Videos of *S. lacertina* were taken in lateral projection, and 5, 10, 17 and 39 breaths were recorded from each of four individuals, respectively.

From the lateral-projection X-ray videos, the areas of the lungs and buccal cavity were measured to indicate air transfer between the atmosphere, buccal cavity and lungs. For analysis, video sequences were digitized using a Radius Video Vision Studio computer board in a Macintosh computer. Video clips were deinterlaced and converted to numbered PICT files with a time resolution of 60 fields s^{-1}. The PICT files were opened in NIH Image, and the density slice tool was used to define a range of densities corresponding to the projected area of the lungs and buccal cavity. Gape was measured as the distance between the tips of the upper and lower jaws.

Pressure

To measure the changes in body pressure during lung ventilation, a Millar Microtip SPR-407 pressure transducer was inserted into the body (pleuroperitoneal) cavity (Brainerd *et al.* 1993). A guide cannula (1.27 mm o.d.) was surgically implanted at a position that was one-third of the distance from the cranial to the caudal end of the body cavity. In order to be certain that the cannula remained secure, we pushed 3 cm of cannula into the body cavity where it rested along the body wall, just internal to the muscle layers. The cannula was then sutured externally to the skin in two or three places. For recording, the transducer was fed down the guide cannula such that the pressure-sensitive tip extended approximately 2 mm beyond the end of the cannula.

Pressures were amplified 100 times through a Tektronix AM502 DC amplifier. The signals were recorded using a GW Instruments data acquisition board in a Macintosh computer running Superscope II software. A color-key graphics overlay (Televeyes Pro, Computer Eyes, Inc.) was used to superimpose real-time pressure traces from SuperScope II onto live video of animals breathing. The combined images were then recorded on video cassette and were used to synchronize pressure waves with kinematics from standard and X-ray video. Pressure waves were also stored digitally, and SuperScope II was used to measure four variables from the pressure wave of each breath: pressure magnitude at the beginning of active exhalation (P_{start}), magnitude at peak pressure (P_{peak}), time from start to peak pressure (T_{peak}) and total duration of increased pressure (T_{end}).

Electromyography

Electromyograms (EMGs) were recorded from the rectus abdominis (RA) and all three layers of lateral hypaxial musculature present in *S. lacertina*: the external oblique (EO), the internal oblique (IO) and the transverse abdominis (TA).

Bipolar patch electrodes were used because they provide electrical insulation between adjacent muscle layers, thus reducing the possibility of cross-talk from other muscles (Loeb and Gans, 1986; Carrier, 1989). Electrodes were fabricated from Dow Corning silastic sheeting (0.25 mm thick) and fine stainless-steel wire (0.051 mm diameter). Two wires were sewn approximately 1.0 mm apart through a section of sheeting cut to 1 cm×1 cm. Approximately 0.5 mm of the wire was exposed and the rest of the wire was covered with additional silicone adhesive. Surgical implantation ensured the correct positioning of the electrodes. A double-sided electrode was used to record the activity of the IO and TA muscles, and was placed between these layers. To minimize surgical trauma, the EO electrode was placed just under the skin, with the recording side facing medially. In general, it is better to place the EO electrode between the IO and the EO, facing out towards the EO, to avoid any possible cross-talk from the IO (Carrier, 1993). In this case, however, our results showed little or no

activity in either the EO or the IO during breathing, and thus cross-talk was not a concern.

Signals were amplified 10 000 times through Grass P511J amplifiers. Spectral analysis (SuperScope II, FFT) of EMG waves from the TA showed the greatest power at frequencies below 200 Hz, with the peak of power at 100 Hz. Therefore, the amplifiers were set to a bandpass of 10 Hz to 10 kHz, and the 60 Hz notch filter was not used. To minimize noise levels, a ground electrode was placed in the water and also in the body of the animal. The signals were digitized using a GW Instruments data acquisition system and Superscope II software. Some signals were recorded at 4000 samples s^{-1}, but since spectral analysis indicated that the frequency of the signals was 200 Hz and below, most signals were recorded at 1000 samples s^{-1}. The waves were digitally filtered using custom-designed filters (WLFDAP, Zola Technologies, Atlanta, GA). Each wave was filtered with a bandstop filter from 50 to 70 Hz to remove line-frequency noise and a lowpass filter to eliminate noise above 300 Hz. EMGs were recorded simultaneously with body cavity pressure, and the onset and duration of the EMG activity were measured with respect to the beginning of the body pressure increase during exhalation. EMG and pressure recordings were made for at least 20 breaths from each of five individual *S. lacertina*, and a single-factor analysis of variance (ANOVA) was used to test for significant differences between individuals. Differences were considered significant at the *P*<0.05 level.

Results

Still frames from an X-ray video (Fig. 2) show the sequence of air transfer between the lungs, buccal cavity and atmosphere in *Siren lacertina*. As the animal lifts its head above the surface of the water, the mouth opens to draw in a small amount of fresh air (Fig. 2, 0.03 s). Exhalation begins as soon as the mouth is open, and the buccal cavity volume remains low and constant during exhalation (0.03–0.79 s). A small amount of air remains in the lungs at the end of exhalation (0.79 s). The breath depicted is typical for the amount of air left in the lungs at the end of exhalation, based on X-ray observations of breathing in four individuals. We were unable, however, to quantify tidal and residual volume because the animals were too long to fit within the X-ray field. We did reposition the animals and record the caudal section of the lungs during breathing, and found that the caudal section collapses more completely than the cranial section.

After exhalation, the buccal cavity expands rapidly to fill with fresh air while the gape begins to close (0.82 s). With the mouth closed, the inflated buccal cavity then compresses to force the air into the lungs (0.86 s, 0.90 s).

In the breath illustrated, the animal expands and compresses the buccal cavity only once. In some breaths, *S. lacertina* takes one or two accessory inspirations after the first expiratory–inspiratory cycle. In these accessory inspirations, the buccal cavity expands and compresses to pump additional air into the lungs, and no exhalation occurs. The number of
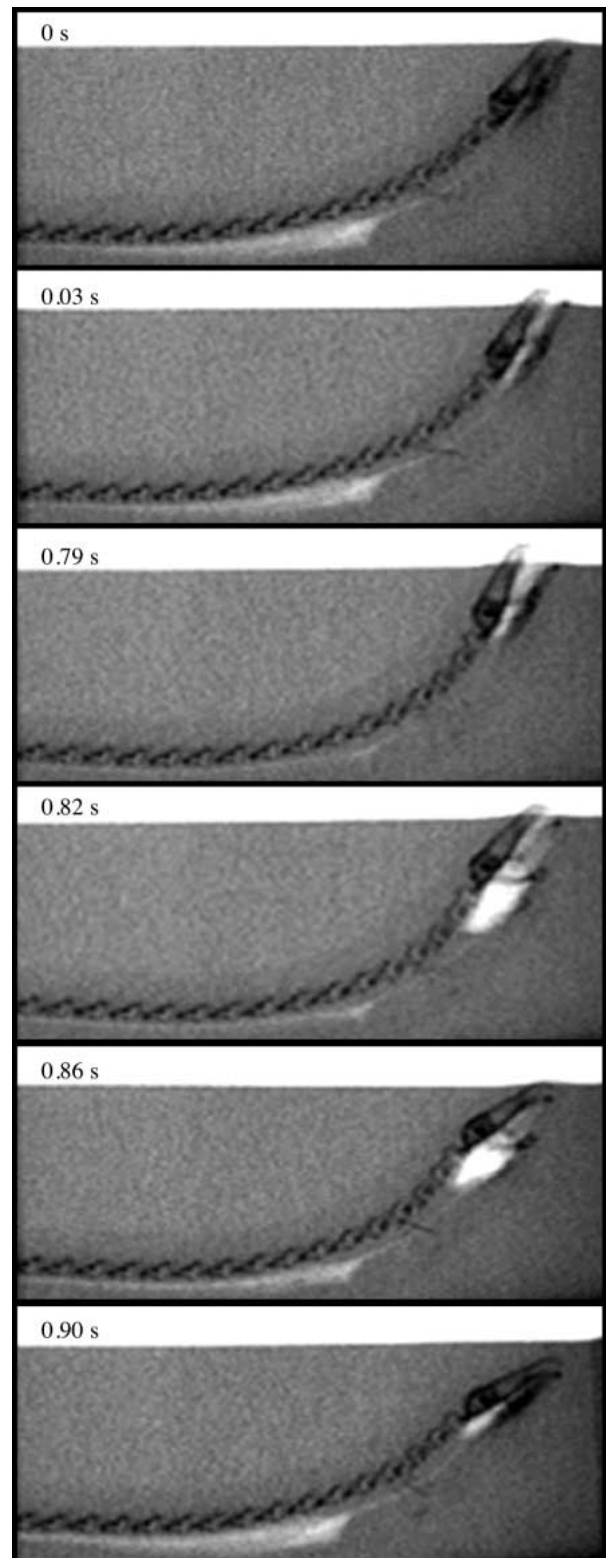

Fig. 2. Still fields from a X-ray video of breathing in *Siren lacertina*. The animal is immersed in water 6 cm deep (gray background) and lifts its head to breathe above the surface (white strip at top of fields). From 0 to 0.79 s, the animal expands its buccal cavity slightly to open an airway and then exhales. From 0.79 to 0.82 s the buccal cavity expands more, and from 0.82 to 0.90 s air is pumped from the buccal cavity into the lungs.

Fig. 3. Body cavity pressure synchronized with kinematics from X-ray video of breathing in *Siren lacertina*. Lung and buccal areas and mouth gape distance were measured from lateral X-ray videos (similar to Fig. 2). Note that as body pressure increases, lung area decreases, indicating that exhalation is at least partially active. In this breath, the animal takes one accessory inspiration, as indicated by the second buccal area peak and associated increase in lung area. For clarity, the pressure wave was filtered with a low-pass filter (20 Hz cut-off) and kinematic plots were smoothed by a three-point running average method. Zero pressure is the ambient hydrostatic pressure at the level of the transducer (approximately 4 cm water depth, 0.4 kPa). Open circles are buccal area, filled circles are lung area and squares are gape distance.
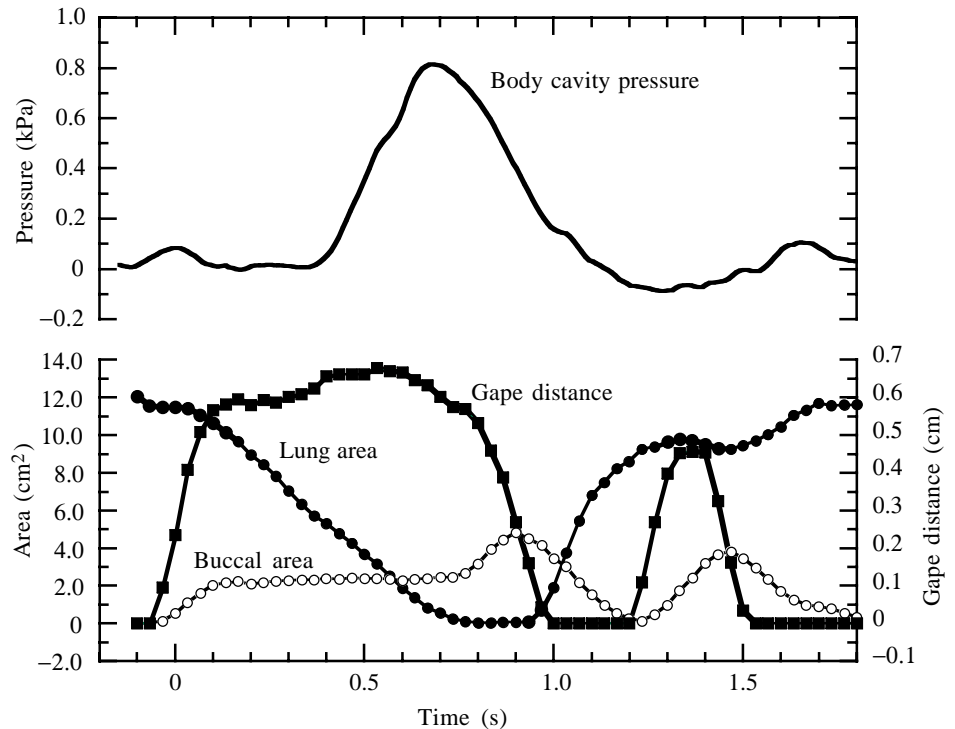
accessory inspirations taken depends on the timing between breaths and how active the animal is. *S. lacertina* takes frequent breaths when active or excited, one breath every 1–2 min, and these breaths generally do not include any accessory inspirations. When at rest, the animals breathe only once every 5–15 min, and these breaths sometimes include one and occasionally two accessory inspirations.

Body cavity pressure and X-ray video

Body cavity pressure was measured in a total of 442 breaths from five individual *S. lacertina*. In every one of these breaths, body pressure increased during exhalation, indicating that the hypaxial muscles contribute to active exhalation in *S. lacertina*.

To determine the exact timing of the pressure increase relative to exhalation, body pressure was recorded synchronously with X-ray video during breathing in three individuals (17 breaths from one individual, 7 from a second, and 5 from a third). Fig. 3 depicts lung and buccal cavity areas (in lateral projection), mouth gape and body cavity pressure for a typical breath. *S. lacertina* begins a breath by opening the mouth widely and partially expanding the buccal area. Reduction in lung area, indicating exhalation, begins immediately after the mouth begins to open, and continues for 0.7 s (mean ± S.E.M. for 15 breaths, 0.52±0.014 s). Body cavity pressure begins to increase at approximately half-way through exhalation, and peak pressure occurs when the lungs reach their minimum volume. Buccal area and gape remain constant during exhalation, and then buccal area increases rapidly to draw in fresh air after exhalation is complete. Gape decreases during this latter buccal expansion, and by the end of buccal

expansion, the mouth is closed. Buccal area then decreases and lung area increases, as air is pumped from the buccal cavity to the lungs. In this breath, a second accessory buccal expansion then occurs after the primary inspiration, and a second mouthful of air is pumped into the lungs (Fig. 3).

Primary inspirations occupied significantly less time when followed by an accessory inspiration (inspiration time is defined as the time during which the lungs are increasing in area). In breaths with no accessory inspiration, the primary inspiration took 0.69±0.026 s (mean ± S.E.M., $N=7$), and in breaths with an accessory inspiration, the primary inspiration took 0.50±0.016 s ($N=8$). Accessory inspirations took 0.60±0.019 s ($N=8$). Not surprisingly, the overall time spent breathing was longer in breaths with an accessory inspiration, 1.83±0.07 s ($N=8$) *versus* 1.40±0.04 s ($N=7$, $P<0.05$; one-way ANOVA).

Hypaxial muscle activity synchronized with body cavity pressure

Our dissections of the hypaxial muscles showed that *S. lacertina* has three layers of lateral hypaxial musculature (in agreement with Naylor, 1978). The muscle fibers of the external oblique (EO) are arranged in an oblique, craniodorsal to caudoventral orientation. The fibers of the internal oblique (IO) run obliquely in a cranioventral to caudodorsal orientation, and in the transverse abdominis (TA), the fibers run at approximately right angles to the horizontal septum.

Electromyograms from the three layers of lateral hypaxial musculature and the rectus abdominis (RA) were recorded synchronously with pressure in the body cavity. Consistent activity associated with breathing was found only in the
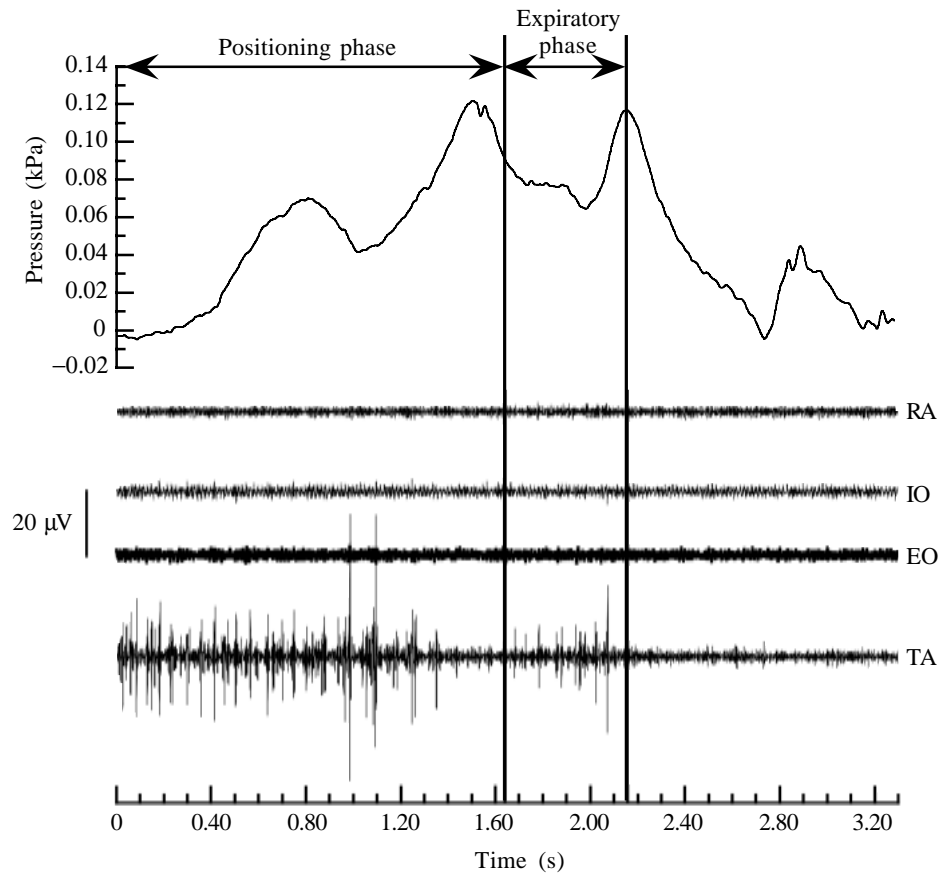
Fig. 4. Body cavity pressure and hypaxial muscle EMGs from one breath in *Siren lacertina*. This breath shows an increase in pressure and transverse abdominis (TA) activity while the animal is lifting its head towards the surface (positioning phase). After a brief silent period, TA activity resumes and body cavity pressure increases during exhalation. No consistent activity was recorded in other hypaxial muscles. Zero pressure is the ambient hydrostatic pressure at the level of the transducer (approximately 4 cm water depth, 0.4 kPa). EO, external oblique; IO, internal oblique; RA, rectus abdominis.

transverse abdominis. Prior to a breath, as the animal positioned itself at the surface, EMG activity was often recorded in the TA (152 out of 225 breaths) and body pressure usually increased (Fig. 4, positioning phase; 217 out of 225 breaths). TA activity then decreased or disappeared altogether, and body pressure declined. After a brief silent period, TA activity increased again, and body cavity pressure increased, this time during the expiratory phase of lung ventilation. At the end of TA activity, body pressure decreased; no TA activity was recorded during inspiration.

No consistent activity was measured during lung ventilation in the EO, IO or RA, although consistent and high-amplitude EMGs were recorded from these layers during undulatory locomotion. In one individual, the EO was active during lung ventilation in seven out of 28 breaths. In these breaths, EO activity began approximately 1 s before the onset of the pressure increase and ended 1.5 s later. In a different individual, IO activity was recorded in six out of 27 breaths. In this case, the IO became active approximately 0.2 s before body pressure began to increase for exhalation, and continued for approximately 0.3 s.

The EMG signals recorded from the TA were lower in both frequency and amplitude than those generally recorded in vertebrate feeding or locomotor muscles. Peak-to-peak amplitudes were in the range 20–40 μV. After digital filtering, our noise threshold was 5 μV, and thus these small EMGs were detectable (Fig. 4). A Fast Fourier Transform was performed

on sections of the signal containing only raw background noise and sections containing both the EMG signal and noise. Above 300 Hz, the power of the combined signal was indistinguishable from that of the noise only signal, and the largest difference between the signals occurred below 200 Hz. A peak in EMG power was often seen at approximately 100 Hz.

Variation in pressure and muscle activity

Body cavity pressure was measured in 217 breaths from five individuals and, in separate experiments, body cavity pressure together with EMG were measured in 225 breaths from the same five individuals. In the pressure only experiments, an increase in body pressure during the positioning phase was seen in 78 % of breaths. In the experiments with EMG, an increase in pressure was seen during positioning in 96 % of breaths, the TA was active during positioning in 68 % of breaths, and the EO was active during positioning in 10 % of breaths.

During exhalation, the TA was always active and body cavity pressure always increased. Four pressure variables were measured during exhalation in each breath (Fig. 5): P_{start}, the pressure magnitude at the end of the positioning phase (beginning of pressure increase during exhalation); P_{peak}, maximum pressure magnitude; T_{peak}, the time from the beginning of the pressure increase to peak pressure; and T_{end}, the time when the pressure returned to baseline (ambient
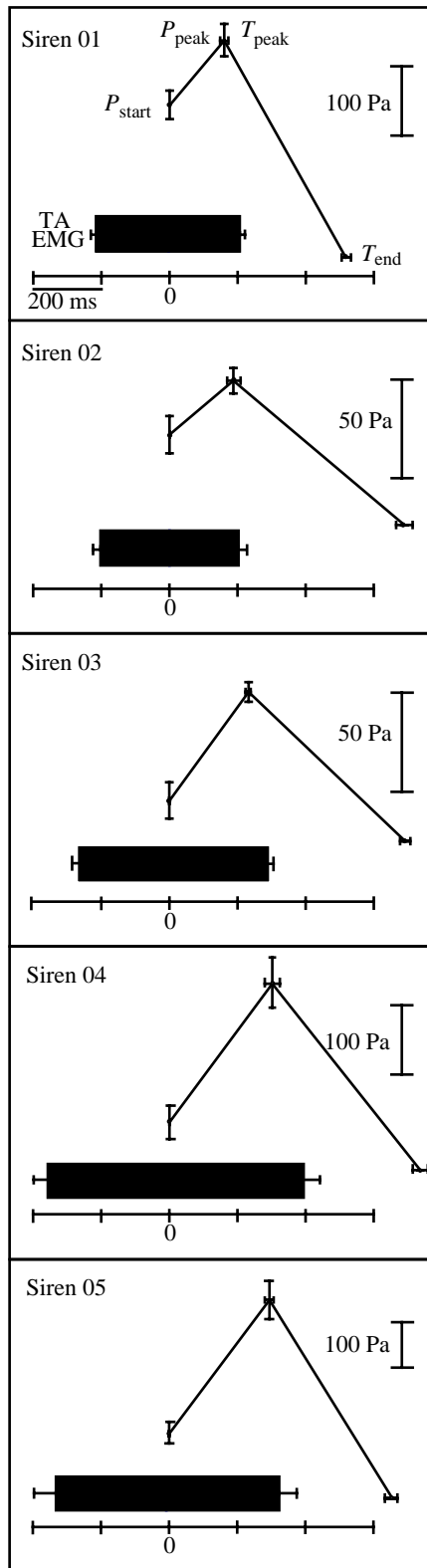
were found in all four pressure variables and two EMG variables (one-way ANOVA; $P<0.05$). Means and standard errors for each individual are depicted in Fig. 5. Variability was relatively small within each individual, but large among individuals. Individuals 01–03 showed relatively smaller increases in P_{peak} over P_{start} than individuals 04 and 05. EMG activity in the TA began 200–400 ms before pressure started to increase (P_{start}) and ended at approximately peak pressure. In individuals with longer T_{peak} (e.g. 04 and 05), TA activity began earlier and lasted longer.

Discussion

The primary goal of this study was to determine whether *Siren lacertina* uses axial musculature for active exhalation. A diagrammatic summary of an air-breath in *S. lacertina* (Fig. 6) shows that the latter part of exhalation is clearly active, as indicated by EMG activity in the transverse abdominis (TA) and an increase in body cavity pressure (phase E2). The TA powers this rise in pressure, which forces air out of the lungs. Other layers of hypaxial musculature, the external and internal obliques and the rectus abdominis, are not consistently active during exhalation, and thus we conclude that the TA is the primary expiratory muscle.

The earlier part of exhalation, in contrast, may be primarily passive (phase E1, Fig. 6). In this phase, the lungs begin to deflate while body pressure remains constant or drops slightly. Passive exhalation may be aided by a combination of hydrostatic pressure and elastic recoil of the lungs. Hydrostatic pressure almost certainly contributes to exhalation, because the lungs of *S. lacertina* are deeper in the water than the mouth during exhalation (Fig. 2), and thus experience a higher pressure. Lung elasticity and smooth muscle in the lungs may also contribute, but measurements of lung pressure would be necessary to confirm this possibility.

Exhalation in phase E1 may not be entirely passive, however, since body cavity pressure is often, but not always, elevated above ambient hydrostatic pressure at the beginning of this phase (Fig. 5 shows the mean P_{start} for each individual at the beginning of phase E1). This elevated pressure appears to be associated with the movement of the animal's head towards the surface of the water. The TA was active during positioning in many, but not all, breaths (Figs 4, 6). Positioning in shallow water, such as the 6 cm depth used in these

hydrostatic pressure at the level of the transducer). Two EMG variables were measured from the TA: onset relative to the beginning of body pressure increase and duration of activity.

Statistically significant differences between individuals

Fig. 6. Diagrammatic summary of breathing in *Siren lacertina*. A qualitative view of changes in body pressure, timing of EMG activity in the transverse abdominis, and the volume of the lungs and buccal cavity, as estimated from two-dimensional X-ray videos. Percentages in the box plots for EMG activity are the actual percentages of breaths in which the transverse abdominis was active during the positioning phase and during expiration. E1, expiration phase 1; E2 expiration phase 2; C1, primary buccal compression; C2, accessory buccal compression. Zero pressure is defined as the ambient hydrostatic pressure.
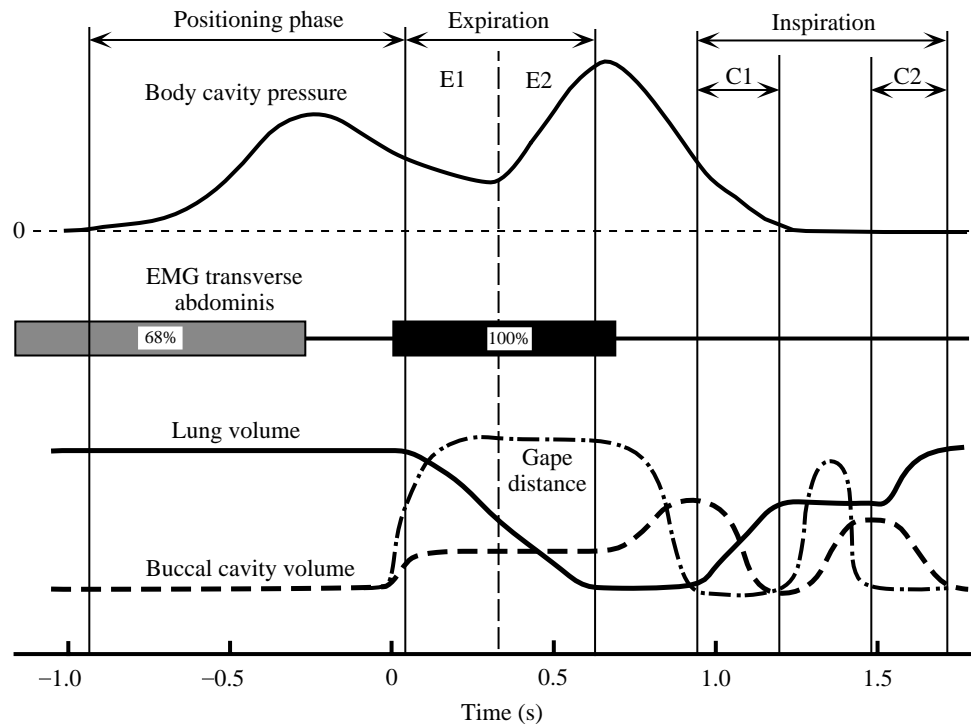
experiments, does not involve any visible swimming movements. Instead, the head rises smoothly to the surface as the cranial region of the trunk bends in a lordotic curve (Fig. 2). This curvature is probably produced by contraction of epaxial musculature, from which we did not record EMGs, and is probably responsible for most of the increase in body cavity pressure during positioning. The TA was active during positioning in most breaths, but other layers of hypaxial musculature were not consistently active. This preparatory activity in the TA may function to shift air towards the front of the lungs, which would increase the buoyancy of the front of the animal and help lift the head towards the surface. It could also serve to stiffen the body and help keep the head positioned at the surface.

Our results suggest that a large proportion of slow or tonic fibers are being recruited in the TA during active exhalation. Activity begins in the TA between 200 and 400 ms before body pressure begins to increase, indicating a slow development of force. Furthermore, EMGs from the TA in *S. lacertina* are relatively low-frequency, with most of their power below 200 Hz. In contrast, TA activity during breathing in *Cryptobranchus alleganiensis* and larval *Ambystoma tigrinum*, recorded by means of the same patch-electrode techniques that we used for *S. lacertina*, had significant power up to 500 Hz (E. L. B., unpublished data). Low-frequency EMGs have also been recorded from the respiratory muscles of *Iguana iguana*, and measurements of isometric contractile properties combined with motor end-plate staining confirmed the presence of tonic fibers in the respiratory muscles of *I. iguana* (Carrier, 1989). Measurements of contractile properties and end-plate staining will be required to confirm the presence of slow and tonic muscle in the TA in *S. lacertina*, but our EMG

results suggest that slow and tonic fibers may contribute significantly to exhalation.

The pattern of buccal expansion and compression in *S. lacertina* is similar to that observed in *N. maculosus* and lepidosirenid lungfishes (*Protopterus aethiopicus* and *Lepidosiren paradoxa*). All of these animals employ a two-stroke buccal pump in which the buccal cavity expands and fills with fresh air before exhalation begins. Air is then exhaled into and through the fresh air in the buccal cavity, and some mixing of expired and fresh air occurs. The buccal cavity is then compressed, and this mixture of air is pumped into the lungs (Bishop and Foxon, 1968; McMahon, 1969; Brainerd *et al.* 1993; Brainerd, 1994). In *S. lacertina* and lungfishes, but not in *N. maculosus*, two discrete phases of buccal expansion serve to reduce the amount of mixing between expired and fresh air (Fig. 6; Brainerd, 1994). Initially, before exhalation begins, the mouth opens and the buccal cavity expands partially, drawing in fresh air from the atmosphere (Fig. 6). This partial expansion opens an airway to the glottis, and then the animal exhales air into and through the fresh air in the buccal cavity. Then, without compressing the buccal cavity to remove the expired gas, *S. lacertina* draws more fresh air into the buccal cavity, and then pumps the mixture of fresh and expired gases into the lungs (C1, Fig. 6). Because much of the buccal cavity expansion occurs after exhalation, less mixing of expired and fresh air occurs in *S. lacertina* and lungfishes than in *N. maculosus*. After this first expiration–inspiration cycle, a second accessory inspiration is sometimes taken in which the buccal cavity expands and compresses again, pumping more air into the lungs (C2, Fig. 6).

The foregoing description of buccal movements differs somewhat from the description in a previous study that

included cineradiographic observations of breathing in *S. lacertina* (Martin and Hutchison, 1979). The studies agree that exhalation precedes inhalation and that 1–3 buccal compressions are used to pump air into the lungs. The studies disagree, however, about what happens during exhalation. In Martin and Hutchison's (1979) observations, *S. lacertina* began to move air from the lungs to the buccal cavity prior to reaching the surface. Martin and Hutchison (1979) also observed two buccal expansions associated with exhalation. In contrast, we never observed exhalation to begin before the animal reached the surface, and only one buccal expansion was ever associated with exhalation.

Our finding that the transverse abdominis is active during exhalation in *S. lacertina* is in agreement with a previous study of lung ventilation in *Necturus maculosus* (Brainerd *et al.* 1993). These results indicate that the presence of an expiration pump in which the TA is the primary expiratory muscle is a primitive character for salamanders (Fig. 1). It is unclear from the literature whether frogs and caecilians also use axial muscles for exhalation (Bentley and Shield, 1973; West and Jones, 1974; MacIntyre and Toews, 1976; Brett and Shelton, 1979; Boutilier, 1984; Vitalis and Shelton, 1990; Carrier and Wake, 1995). Frogs and caecilians, however, have highly derived body musculature. Frogs have only two layers of lateral hypaxial musculature (the primitive condition is four layers), and caecilians have modified their axial musculature for concertina locomotion (Naylor and Nussbaum, 1980; Nussbaum and Naylor, 1982; O'Reilly *et al.* 1997; Summers and O'Reilly, 1997). Thus, it would not be surprising to find that the hypaxial musculature has lost its expiratory function in frogs and caecilians.

In the same manner as salamanders, amniotes also use the TA for active exhalation (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). Given that salamanders have a more primitive body form than anurans and gymnophionans, and share the use of the TA for exhalation with amniotes, we conclude that the expiratory function of the TA is primitive for tetrapods (Fig. 1). This conclusion supports the idea that aspiration breathing evolved in two steps: first from the use of a pure buccal pump, with no contribution from the axial musculature (as in lungfishes), to the use of hypaxial musculature for exhalation and a buccal pump for inhalation (as in urodeles) and, second, to the use of the axial musculature for both exhalation and inhalation (as in amniotes).

It is unclear how these neontological data relate to paleontological data on the early amphibians. Unlike modern amphibians, which are small animals with short peg-like ribs, the earliest known fossil amphibians were large and many had well-developed ribs. It is possible that some of these early tetrapods used their ribs for costal aspiration, but some probably did not. *Icthyostega* had broad, overlapping ribs that do not seem suitable for aspiration, and many Paleozoic amphibians are thought to have had quite short ribs (Carroll,

1988). Furthermore, all of the early amphibians had the broad, flat heads that are typical of buccal pumpers.

Modern amphibians (Lissamphibia) are thought to have evolved by paedomorphosis from a lineage of large (>1 m long) Paleozoic amphibians, the Temnospondyli (Bolt, 1977). If temnospondyls used the ribs for aspiration, then this function has been lost in lissamphibians. One possible scenario is that the larvae of temnospondyls breathed in the same manner as modern salamanders and that this larval breathing mechanism of an expiration pump combined with a buccal pump has been retained through paedomorphosis in modern amphibians.

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