

Lung collapse among aquatic reptiles and amphibians during long-term diving

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Abstract

Numerous aquatic reptiles and amphibians that typically breathe both air and water can remain fully aerobic in normoxic (aerated) water by taking up oxygen from the water via extrapulmonary avenues. Nevertheless, if air access is available, these animals do breathe air, however infrequently. We suggest that such air breathing does not serve an immediate gas exchange function under these conditions, nor is it necessarily related to buoyancy requirements, but serves to keep lungs inflated that would otherwise collapse during prolonged submergence. We also suggest that lung deflation is routine in hibernating aquatic reptiles and amphibians in the northern portions of their ranges, where ice cover prevents surfacing for extended periods.

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1. Introduction

Many aquatic reptiles and amphibians with lungs exchange gases with both air and water. The extent of partitioning between aerial and aquatic modes for animals in normoxic water differs for O₂ and CO₂, with more CO₂ typically being lost to the water than O₂ taken up, because of the higher solubility of CO₂ and the greater ease with which it diffuses through integumentary, gill, and other surfaces used for aquatic gas exchange. The partitioning for each gas will depend on a number of factors, including temperature, metabolic rate, body size, and presence of specific structures for aquatic gas exchange (gills, skin foldings, vascularized buccal and cloacal cavities, etc.).

Lunged aquatic ectotherms can be obligate or facultative air-breathers, depending on the conditions mentioned above. However, one observation seems to hold for all such bimodal breathers: They breathe air occasionally, even when it can be demonstrated that there is no requirement that they do so from a gas exchange viewpoint. A number of studies have shown that aquatic ectotherms that routinely breathe air can survive for extended periods without air access when submerged in normoxic water, including most species of lunged aquatic salamanders (Guimond and Hutchison, 1976; Ultsch 1973; Wakeman and Ultsch, 1976) and lunged tadpoles (Crowder et al., 1998; Feder and Wassersug, 1984; Proynch and Wassersug, 1994; Ultsch et al., 1999). In addition, many species that would drown rather quickly (e.g., in traps left submerged overnight) at summer water temperatures can become completely aquatic gas-exchangers during hibernation at low temperatures in normoxic water, accumulating no lactate or CO₂. Examples include frogs (Pinder et al., 1992; Stewart et

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al., 2004; Ultsch et al., 2004) and some species of turtles (Ultsch, 1988; Crocker et al., 2000; Reese et al., 2001, 2003).

Prolonged diving, however, should inevitably result in loss of all lung gases in these ectotherms, and presumably to lung collapse, for the same reasons that the compressible gas gills of insects lose volume during a dive and eventually dissipate (Rahn and Paganelli, 1968; Mill, 1974; Chaui-Berlinck et al., 2001). For the gas gill, a decrease in volume will occur by hydrostatic pressure when the animal dives. Some O₂, and therefore volume, will be lost initially from the bubble to the water due to the increase in bubble PO₂ resulting from the hydrostatic pressure, followed by a further decrease as O₂ is removed from the bubble faster than it can diffuse in. The falling PO₂ will result in an increase in PN₂ to greater than that of the water, and N₂ will then diffuse into the water and the bubble will dissipate. In the diving ectotherm, there will be little or no initial O₂ loss to normoxic water, and the N₂ will take longer to diffuse to the water because it must pass through the animal, but the result must be the same. In fact, several authors have noted this effect. Ultsch and Jackson (1982), for example, found that turtles (*Chrysemys picta bellii*) submerged for 5–6 months at 3 °C in normoxic water had collapsed lungs, while those submerged in N₂-equilibrated water had gas in their lungs, and Feder and Wassersug (1984) reported that tadpoles (*Xenopus laevis*) submerged in normoxic water at 25 °C lose their lung gases in about an hour.

Here we show that a variety of tadpoles and adult and larval salamanders, all of which breathe air if it is accessible, lose the gas from their lungs when submerged in normoxic water for extended periods, and that the time for complete loss varies among species and with body size, but does not exceed a few days at room temperature. All of the species used can survive prolonged (at least weeks to months) submergence in normoxic water, and we suggest that air breathing under these conditions occurs primarily to prevent lung collapse, with gas exchange and buoyancy being secondary considerations.

2. Materials and methods

2.1. Animals

The amphibians used were either field-collected or purchased from commercial suppliers. Species included bullfrog tadpoles (*Rana catesbeiana*), larval salamanders (*Ambystoma tigrinum*), mudpuppies (*Necturus maculosus*), and sirens (*Siren lacertina*). They were maintained in aquaria in well water aerated over marble chips at 20–25 °C and fed with food appropriate to the species. All animal care and use was governed by protocols

approved by the Institutional Animal Care and Use Committee of the University of Alabama.

2.2. Submergence protocol and lung inspection

Animals with prior air access were prevented from surfacing by a plastic grating placed at about mid-depth in their tank. The water above the grating was continuously aerated, which provided mixing and maintained the water PO₂ near 155 mm Hg (normoxic). When animals were periodically removed for dissection, transfers were made underwater to prevent breathing. The animals were then deeply anaesthetized with either urethane or MS-222 (tricaine methanesulfonate) and pithed. The lungs were dissected away from the body cavity underwater. It was noted if they floated upwards in the body cavity and if they floated upon removal with the trachea attached. The tips of the lungs were then cut underwater, and the lungs were pressed and rolled flat to determine if there was any gas remaining.

2.3. Rate of lung gas loss in *Siren lacertina*

We used two methods to determine the rate of lung gas loss in submerged *S. lacertina*. First, an animal was placed inside a rigid plethysmograph constructed from an aluminum block (internal dimensions 30×6×4.5 cm) with a 1-cm-thick acrylic cover fastened securely with 36 bolts. A three-way stopcock embedded in the cover was connected to a Statham Model P23D pressure transducer connected to a Grass Model 7D polygraph. Volume lost from the lungs created a negative pressure, which was equalized by addition of water through a second embedded stopcock. We assumed that the water added equaled lung volume lost. Secondly, we submerged a siren in normoxic water and used X-ray videography to monitor the changes in lung volume, using the methodology of Brainerd and Monroy (1998). We then transferred the X-ray images from digital video tape to a computer and processed the images in Adobe Photoshop 8.0 in order to increase contrasts among lung gas, skeleton, and tissue.

3. Results

3.1. Bullfrog (*Rana catesbeiana*) tadpoles

Several experiments were conducted with bullfrog tadpoles, which were staged according to Taylor and Kollros (1946). For reference, stages I–V are early hindlimb bud stages, stages VI–XIX exhibit progressive hindlimb development, forelimbs emerge at stage XX, internal gills are lost at stages XXII–XXIII, and stage XXV is a young metamorph. Tadpoles of all stages with air access always had gas-filled lungs that floated upon dissection. Lungs dissected from five tadpoles (stages I–VII) submerged in normoxic water at 23–25 °C floated and contained gas after 6 h, but those of stages II–XV

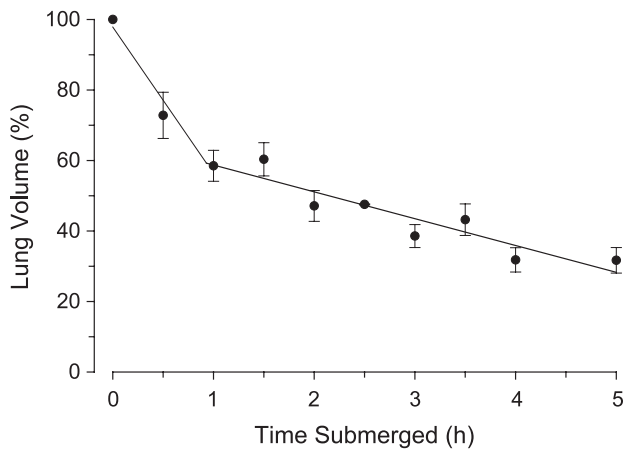


Fig. 1. Decrease in original lung volume with time for *S. lacertina* submerged in normoxic water at 23–25 °C. Extrapolation of the slow phase of deflation predicts zero volume at 8.74 h.

sank and were gas-free after 12 and 24 h. The lungs appeared reduced in volume but did not appear visibly flattened. Five tadpoles kept submerged for eight days were then allowed access to air (stages XII–XXIII); after 4 days, the lungs were found to be re-inflated and

appeared similar to those of normal tadpoles with access to air.

To test whether air breathing is required for continued development toward metamorphosis (development of fore and hind limbs, tail regression, etc.), three groups of 10 tadpoles each at different stages of development (V–XII, XIV–XIX, and XX–XXII) were submerged in normoxic water at 22–23 °C. The animals fed and developed normally in all groups, based only upon external features. Death occurred at stages XXII or XXIII, coincident with the time of regression of the internal gills.

3.2. Mudpuppies (*Necturus maculosus*)

Mudpuppies (63–124 g) in normoxic water of about 30 cm depth at 23–25 °C were observed to occasionally surface and upon dissection always had gas-filled lungs. When submerged in normoxic water for 24 and 48 h, the lungs of each of two individuals floated upon dissection and contained gas. At 72 and 96 h, dissected lungs sank, contained no gas, were visibly flattened, and did not appear to contain excess fluid. Animals submerged for 30

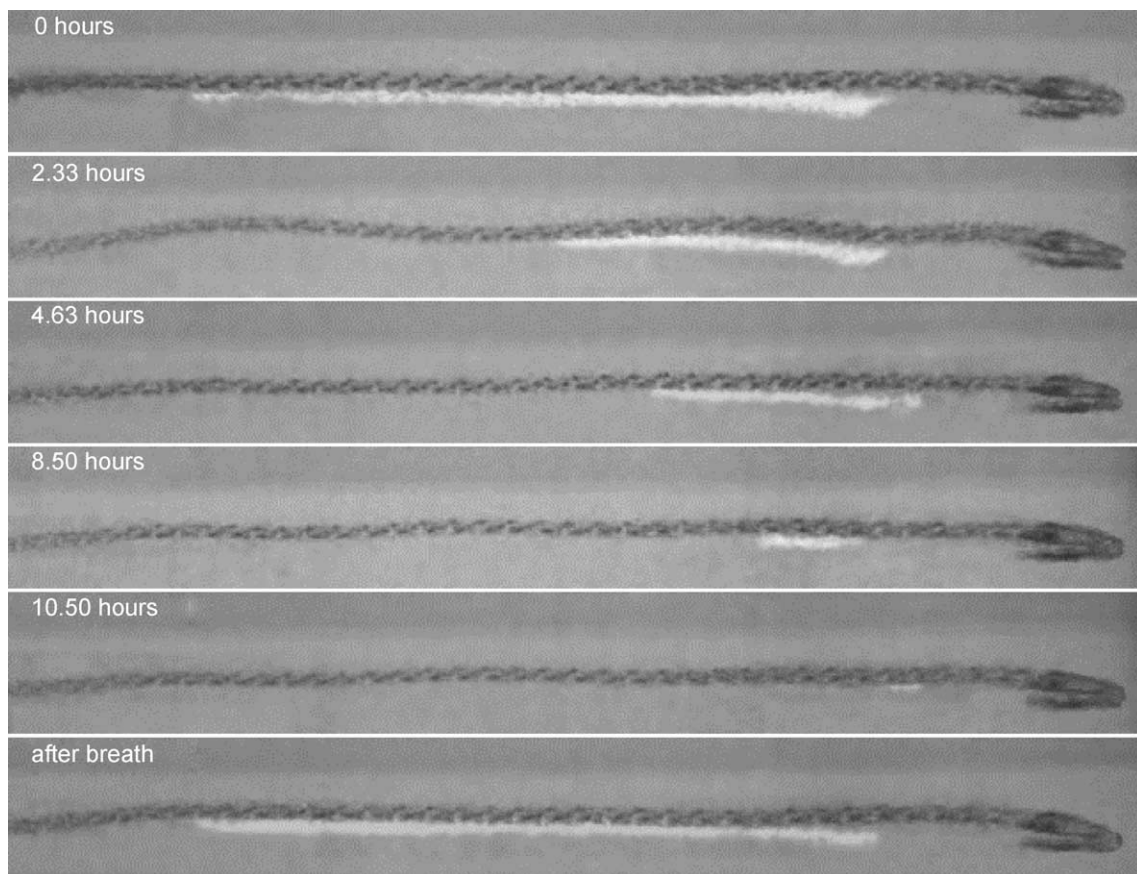


Fig. 2. X-ray videography of the loss of lung gas from a 20 g *S. lacertina* submerged in normoxic water at 20–21 °C. These are X-ray positive images in which lung gas appears as a whitish area ventral to the dark gray vertebral column. A small amount of gas is visible at the center of the right half of the panel at 8.5 h, and very little is visible at 10.5 h. When the animal was allowed access to air, it took an air breath almost immediately and re-inflated the full length of its lungs (last image, after breath).

days had lungs that appeared similar to those at 3 and 4 days—no gas, flattened, and containing no excess fluid. Mudpuppies ($n=1-2$) allowed air access for a week after 4, 5, 6, 7, and 8 weeks of submergence all re-inflated their lungs, which appeared similar to those of air-access controls.

3.3. Tiger salamander larvae (*Ambystoma tigrinum*)

Lungs dissected from two tiger salamander larvae (92 and 100 g) that had air access at 23–25 °C floated and contained gas. Lungs from animals submerged in normoxic water for 24 and 48 h also floated and contained gas, but those from animals submerged for 72 and 96 h sank and were devoid of gas (two at each time, 76–148 g). Two larvae (76 and 92 g) submerged for 4 days and then allowed air access for a week had re-inflated their lungs, which appeared similar to those of control animals.

3.4. Sirens (*Siren lacertina*)

Large *Siren* (>700 g) will drown if kept forcibly submerged in normoxic water at 23–25 °C, so experiments were conducted with smaller animals. Two animals were submerged in normoxic water for 1 week, and did not appear distressed. One (452 g) removed had flattened and gas-free lungs. The second was allowed air access for a week, and the lungs were re-inflated to a normal appearance.

The decrease in lung volume of eight sirens (16.1–55.7 g) as determined by plethysmography exhibited a two-phase response (rapid loss of O₂ due to uptake by the animal, followed by a slower diffusive loss of N₂; Fig. 1). We used the method of Yeager and Ultsch (1989) to find the two best-fit regression lines, which intersect at 0.93 h. The y -intercept of the slow phase of lung volume decrease is 8.7 h, which is the predicted time for complete loss of lung gas. This estimate is in good agreement with X-ray videography at 20–21 °C of a 20 g siren, which showed little lung gas left by 8.5 h and almost none by 10.5 h of submergence, followed by re-inflation of the entire length of the lungs upon air breathing (Fig. 2). In three X-ray video observations of re-inflation in the same individual after 7–12 h of submergence, the first two or three air breaths did not appear to re-inflate the lungs to their initial volume (compare the diameter of the anterior part of the lungs in the first and last images in Fig. 2); however, we assume complete re-inflation would occur during subsequent breathing episodes.

4. Discussion

A number of species of aquatic reptiles and amphibians that are bimodal gas exchangers (air and water

breathers) do not require air breathing to meet their routine gas exchange requirements, even at temperatures at which they are normally active, if the water is normoxic. Yet, they all breathe air occasionally, even such animals as hellbenders (*Cryptobranchus alleganiensis*), which are predominantly skin breathers, and mudpuppies (*Necturus*), which utilize their skin and have efficacious external gills (reviewed by Guimond and Hutchison, 1976). The simple structure of the lungs of these two species has led some to suggest that the lungs are primarily buoyancy-control organs (Guimond and Hutchison, 1972, 1973). However, both of these species are bottom dwellers, and hellbenders are typically found in fast-moving mountain streams and rivers, as are some smaller species of *Necturus*, where buoyancy is not an issue, and may be a disadvantage. For example, darters (e.g., the fish genus *Etheostoma*) live in such habitats, and they have lost the gas bladder, and the plethodontid salamanders, some of which inhabit streams, have no lungs. In addition, Ultsch and Duke (1990) have shown that when hellbenders and mudpuppies are kept at 20 °C in severely hypoxic water (9–10 mm Hg), they can survive for at least 5–11 days by air breathing, indicating that the lungs, however simple in appearance, can be functional gas exchange organs.

The question of why some amphibians breathe air if it is not necessary for respiration has been addressed by Feder (1984), who recognized the obvious advantages of air breathing in hypoxic waters. He suggested that air breathing may simply be a matter of precocial development of the lungs, which will become the major gas exchange organ in the adult, and for use in buoyancy control in the few aquatic amphibians that are not, or not always, bottom dwellers (e.g., *X. laevis* larvae, and *A. tigrinum* larvae under some circumstances). We suggest that amphibian larvae in normoxic water, as well as facultative air-breathers such as *Siren*, *Necturus*, and *Cryptobranchus*, breathe air in order to keep their lungs inflated, and therefore ready for use in case of aquatic hypoxia, however infrequent that may be. Furthermore, there is evidence that for tadpoles, air breathing is required for normal lung development, growth, and successful metamorphosis (e.g., *X. laevis* larvae, Proynch and Wassersug, 1994).

Another question is whether lung collapse occurs naturally due to prolonged submergence, and if it does, can the animals re-inflate their lungs without damaging them? In our experiments, re-inflation was possible after up to 8 weeks of submergence, at least for *Necturus*. We presume this to be the case in north-temperate climates where ice covers the habitat for months. A number of species of aquatic turtles, frogs, and salamanders hibernate for 4–7 months in ice-covered habitats, and their lungs must collapse during the winter. A few species, such as *Siren* and *Amphiuma*, may collapse, or collapse portions of, their lungs upon exhalation (Martin and Hutchison, 1979; Daniels et al., 1994; Brainerd and Monroy, 1998). Thus,

lung collapse certainly does occur, at least seasonally, in a number of species.

Whether lungs are collapsed briefly or for long periods, re-opening is facilitated by surfactant (Daniels et al., 1995). Daniels and Orgeig (2001) have noted three important comparative aspects of lung surfactant: (1) the surfactant system is highly conserved among air-breathing vertebrates, (2) temperature is a major controller of surfactant composition, and (3) an anti-adhesive (anti-glue) function of surfactant is primitive and ubiquitous among the vertebrates. Long-term underwater hibernation is certainly a case when the anti-glue function of surfactant would be paramount.

In summary, we suggest that air breathing among aquatic facultative air-breathers occurs in order to prevent inevitable lung collapse, that collapse does occur routinely among overwintering aquatic reptiles and amphibians that spend long periods under ice, and that surfactant plays a major role in facilitating re-inflation of the lungs after prolonged closure.

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