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EMBRYONIC AND LARVAL RESPIRATION IN THE ARBOREAL FOAM NESTS OF THE AFRICAN FROG *CHIROMANTIS XERAMPELINA*

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Summary

In Zimbabwe, female *Chiromantis xerampelina* construct spherical foam nests that are suspended above temporary water. The nests average 624 ml in volume and contain 854 eggs. The 1.7 mm ova have exceptionally thin jelly capsules and are dispersed in the foamy core of the nest, which is surrounded by a layer of eggless foam. At 25 °C, each embryo requires 3.5 days to reach hatching at developmental stage 22, during which it consumes 30 μl of oxygen. After hatching, each larva remains in the nest for 2 more days and consumes a further 123 μl of oxygen. The fresh foam contains 77 % air, which is sufficient to supply all of the oxygen requirements of the embryos until well after they hatch. Therefore, the size of the egg mass is not limited by oxygen availability as it is in many other anurans. Oxygen also diffuses into the nest from the atmosphere, but the rate is severely restricted by the wet foam, despite the presence of bubbles. Drying of the outer layer of foam greatly increases its oxygen conductance, but the larvae remain in the inner core of wet foam, where they compete for oxygen at the periphery. With further drying of the nest, the wet foam diminishes in volume and concentrates the larvae at a time when their oxygen demands are approaching the maximum. Oxygen pressures within the wet foam drop below 10 kPa and oxygen uptake by the larvae becomes progressively limited, possibly stimulating their emergence from the nest. The delay between hatching and escape from the nest permits the larvae to grow and mature to a stage at which all of the clutch can emerge simultaneously.

Introduction

Amphibian eggs develop aerobically, obtaining oxygen from the environment through the vitelline membrane and jelly capsules that surround them. Although these barriers may appear small, they can create substantial resistances to diffusion. For example, oxygen uptake by the single eggs of the terrestrial-breeding frog *Pseudophryne bibronii*

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can be limited by diffusion, although the jelly capsule is less than 1 mm thick and the maximum rate of oxygen consumption is approximately 1 μl h^{-1} (Seymour *et al.* 1991). Furthermore, if an egg is surrounded by other eggs in a large clutch, the outer embryos may intercept the oxygen diffusing into the egg mass and prevent the inner embryos from developing. The aquatic egg masses of the frog *Limnodynastes tasmaniensis*, for example, must be constructed such that all embryos are less than about 13 mm from the free water to ensure that they are adequately oxygenated (Seymour and Roberts, 1991). It is also advantageous for aquatic egg masses to be suspended in the water, rather than resting on the bottom, because oxygen can approach the eggs from all directions and the surface water is often better oxygenated (Moore, 1940). Terrestrial egg masses are assured a constant environmental oxygen level, but they face other problems. In addition to the danger of desiccation, the oxygen supply can be limited. Surface tension and gravity cause gelatinous egg capsules to collapse on one another and the egg mass sticks to the substratum. Gas spaces between the eggs may disappear and oxygen may be available only from above. Consequently terrestrial egg masses are often small or thinly spread (Salthe and Duellman, 1973).

One adaptation to both aquatic and terrestrial breeding in amphibians is the construction of foam nests in which eggs are deposited in a mass of jelly that is whipped into a froth by the frog. Foam-nesting has apparently evolved several times and occurs in at least six families (Rhacophoridae, Leptodactylidae, Myobatrachidae, Hylidae, Microhylidae and Hyperoliidae) (Hödl, 1990, and personal communication; Haddad *et al.* 1990). In aquatic breeders, the function of the foam is to suspend the eggs in better-oxygenated water, and in terrestrial breeders it is usually considered advantageous in protecting the egg mass from desiccation, insulating it from temperature fluctuations and defending it against predators (see Seymour and Roberts, 1991, for references). The foam of terrestrial nests is also advantageous, as it allows the deposition of a clutch much larger than would be possible were the eggs to be surrounded by jelly alone. The bubbles in the foam not only facilitate oxygen diffusion into the egg mass (diffusion in air occurs over 250 000 times faster than in jelly) but also provide a capacious oxygen store for immediate use by embryos, without the need for diffusion over long distances. As the respiratory role of foam has not been adequately considered in the literature, we here quantify the oxygen balance in the large foam nests of the African rhacophorid frog *Chiromantis xerampelina* Peters.

The frogs breed during the summer rainy season and construct nests in trees and other substrata above temporary pools (Wager, 1965). Nest construction has been documented recently by Jennions *et al.* (1992). The female produces a thick mucoid fluid from her cloaca and whips it into an elastic froth with her hind legs. One or more males fertilize the eggs, which are incorporated into the foam as they are laid. On the following night, the female returns to the nest and adds a layer of eggless foam to the outside of it, presumably to protect the eggs from desiccation during development (M. D. Jennions, personal communication). Arboreal foam nests are also produced by the other two species of the genus, *C. rufescens* and *C. petersii* (Coe, 1974). The nests of *C. xerampelina* are reported to reach 20 cm in diameter (Wager, 1965) and contain several hundred moderately sized eggs (Jennions *et al.* 1992). Rates of oxygen utilization within these large nests, which are

incubated out of water at relatively high temperatures, would be expected to be high. Adequate oxygen supply solely by diffusion from the environment would be impossible in a globular jelly egg mass without foam, so the bubbles are vital to the fecundity of this species. This study analyses the adaptive role of the foam in embryonic respiration and survival by documenting the structure and composition of the nest, the distribution of embryos and larvae, their rates of oxygen demand and the resulting profiles of oxygen tension within the nest. Changes in these features are followed throughout embryonic and larval development until the tadpoles leave the nest.

Materials and methods

Collection of nests

Fresh foam nests of *C. xerampelina* were located during December 1992 and January 1993 in two areas within Zimbabwe: associated with temporary streams in grazing land near Chegutu (30˚20′ E, 18˚5′ S, altitude 1200 m), and over muddy pools near the Rukomeshi River at the base of the Zambezi Escarpment (29˚24′ E, 16˚8′ S, altitude 500 m).

The dimensions of single nests were measured with callipers to ±5 mm and the nests were sketched. Nest temperatures (±0.2 ˚C) were measured with a Fluke thermocouple thermometer on several nests during the day at Chegutu and long-term temperature fluctuations within two field nests at Rukomeshi were monitored with a Grant data-logger.

Undisturbed nests were obtained if they were attached to leaves or small branches which could be cut. Nests attached to large branches, earth or stone were freed from the substratum and placed individually on a flat glass plate, over a 10–15 mm hole in the centre. The hole was closed from below with a greased glass slide that could be temporarily removed to allow access to the centre of the nest. A total of 21 nests from Chegutu were transported to a laboratory at the University of Zimbabwe in Harare. Although removal and transport usually broke the outer layer of foam, the shape of the nest was retained, and after resting for a day on the horizontal glass, the outer foam dried and adhered to it, permitting the glass to be turned vertically for the duration of incubation. The nests were kept in a constant temperature room at 25±1 ˚C and humidity of approximately 60 %.

Morphology of nests, embyros and larvae

The structures of two fresh nests were determined by freezing them and slicing them horizontally and vertically. Composition and dimensions of nests in which the larvae had hatched, but had not emerged from the foam, were measured by two methods. First, the distribution of hatched larvae in wet foam was measured in three nests attached to glass. We traced the vertical distribution directly through the glass prior to larval escape, and the horizontal outline of the nest cavity from serial, 10 mm sections of the dried foam obtained after the nest had been vacated. In the second method, four nests were opened and the larvae and wet foam were scraped into a tared glass vial, in which the volume and mass of the combined larvae and foam were measured. Assuming that the density of the

larvae and foam was 1.05 g ml^{-1}, the volume of air in the wet foam was estimated. In both cases, the live larvae were counted, and a sample containing 50 larvae was separated from the foam and weighed. The yolky guts were removed from a subsample of six hatchling larvae and these gut-free larvae were weighed, dried and reweighed.

Embryos and larvae were obtained from the nests, either through a small hole cut in the foam and plugged afterwards with dry foam from another nest, or through the hole in the glass plate. Embryonic development was 'staged' according to Gosner (1960) between the earliest stage observed (Stage 8) and that when the larvae escaped from the nest (Stage 22). Developmental peculiarities that differed from Gosner's system were observed at Stages 15 (no rotation of the embryos occurs, although cilia are present; a narrow neural fold is definitive), 17 (tail bud grows as long as the diameter of the yolk before the muscular response of Stage 18 appears) and 21 (transparency of cornea is difficult to observe, so gross flattening of the tail is definitive). Stage 22 was indicated by tail-fin circulation. After Stage 22, development of the operculum and limb buds was extremely retarded relative to Gosner's system. Further development was documented by changes in the length of the tail in relation to total length. When viewed from above in a dissecting microscope, the beginning of the tail was defined as the point at which the yolky gut disappears under the tail. An ocular micrometer, accurate to 0.01 mm, was used to measure the larvae. A linear regression of relative tail length against time was made for larvae from four clutches that had been incubated from Stage 10 at a constant 25 °C.

Oxygen consumption measurements

Oxygen consumption rates ($\dot{V}_{O_2}$) were obtained from embryos and larvae with four oxygen uptake chambers (volume approximately 0.67 ml; no. 1271, Diamond General Corporation, Ann Arbor, Michigan, USA) fitted with miniature Clark oxygen electrodes (Diamond General no. 730). Electrode current was measured with a picoammeter (Diamond General no. 1231). The polarizing voltage (−0.75 V) was maintained between measurements with a custom-built switching box. The electrodes were calibrated with a sodium sulphate–borax P_{O_2}-zero solution and air-equilibrated water. Electrode current was less than 1.5×10^{-8} A, and the oxygen consumption in empty chambers was negligible. Chamber temperature was maintained at 25 °C (±0.02 °C) by a thermocirculator (no. F4391, Haake, Berlin, Germany). One or two eggs were placed in each chamber, depending on developmental stage. Isothermal, air-equilibrated distilled water was flushed through the chamber at the beginning of each experiment, and P_{O_2} was allowed to decrease. To limit disturbance of the animals, the water in the chamber was stirred with a small magnetic bar only during the 15 s period immediately preceding each reading. The current produced by air-equilibrated water was measured before and after each experiment, and $\dot{V}_{O_2}$ was calculated from the oxygen capacitance of water, the water volume in the chamber and the electrode current (corrected for electrode drift, which was assumed to be linear).

Oxygen levels inside ten foam nests were measured with two types of Clark microelectrodes. Rough measurements on nests containing late-stage larvae were made by inserting a Diamond General no. 731 electrode into the pool of larvae and moist foam through the hole in the glass plate or through a hole in the dried foam. Precise

measurements were obtained by using a micromanipulator to insert a Diamond General no. 737 Clark electrode (tip diameter 0.15 mm) into nests on the glass plates. The electrode tip was advanced through the foam, rather than through the hole on the glass plate, to avoid leakage of air through the hole. Punctures in the foam caused by the electrode were sealed with foam from another nest.

Statistics

Results are given as means and 95 % confidence intervals, and sample size, *N*, refers to number of clutches, unless stated otherwise.

Results

Structure of the nest

Nests were attached to diverse substrata, including the leaves, twigs, branches, trunks, and roots of trees and grasses, and also bare rock and earthen banks. However, they were always attached to the vertical face or underside of the substratum, and always overhung temporary water. The height of the nest above the water varied from 4.5 cm to over 2 m. Temperatures in nine nests at Chegutu averaged 25.3±2.4 ˚C at time of collection. At Rukomeshi, temperatures in two nests averaged 25.9±0.3 ˚C over 2 days.

The dimensions of 10 complete nests (with outer eggless foam) are given in Table 1. Because the shape of a nest approximated a sphere with one flat side attached to the substratum, nest volumes were estimated according to the equation: $V=(4/3\pi r^3)(d/2r)$, where V is the volume (ml), r is half of the mean of height and width (cm) and d is the depth of the foam to the substratum (cm). The mean volume of 624 ml represents an effective radius of 5.3 cm, if the nest were a perfect sphere. The volume of two incomplete nests (without outer eggless foam) averaged 508 ml.

Two 25 ml samples of foam from a fresh nest were weighed and dried at 65 ˚C. Any eggs found in the dried foam were weighed separately and their mass subtracted from that of the dried foam. The density of the eggless wet foam was 0.21 g ml^{-1} and that of the dry foam was 0.0023 g ml^{-1}. Assuming a density of the jelly mucopolysaccharide and mucoprotein of 1 g ml^{-1}, air accounted for 79 % of the foam volume of the fresh nest, increasing to 99.8 % when the foam dried. The thickness of the dried foam was measured over the undissolved lateral and upper surfaces of eight nests after the larvae had escaped. The thickness ranged from 10 to 28 mm and averaged 19.7 mm (CI=1.4 mm). Thus, dried foam accounted for about 75 % of the final nest volume.

Before hatching, the eggs remained at the same location in a single compartment of moist foam, averaging 155 ml in volume, inside the shell of dried foam (Fig. 1, Table 1). The outermost eggs were killed if the foam dried around them. There was no obvious change in the structure of the moist foam until hatching, when the perivitelline liquid was released from the eggs, softening the foam. The larvae were then able to swim among the bubbles. The volume of moist foam decreased as some of the gas in the bubbles was lost to an air space that formed above the pool of foam. As the air space volume slowly increased, the larvae congregated in a foamy pool at the bottom of the nest cavity. During the 2 days after hatching, this pool gradually descended, dissolving previously dried foam

Table 1. *Characteristics of the arboreal nests and larvae of* Chiromantis xerampelina *on the day of escape from the nest*

Variable	Mean	95 % CI	*N*
Whole complete nests			
Height (cm)	11.72	1.19	10
Width (cm)	11.29	1.52	10
Depth (cm)	8.18	1.93	10
Volume (ml)	624	286	10
Equivalent radius* (cm)	5.3		
Larvae			
Number live	695	165	12
Wet body + gut mass (mg)	5.02	0.82	8
Wet body mass (mg)	3.05	1.24	5
Dry body mass (mg)	0.45	0.09	5
Total wet mass* (g)	3.49		
Wet gut (yolk) mass (mg)	1.08	0.05	5
Dry gut (yolk) mass (mg)	0.59	0.14	5
Wet foam and larvae			
Volume (ml)	48.13	15.1	7
Larval density (number ml^{-1})	15.72	4.04	7
Fractional air space (ml ml^{-1})	0.77	0.04	4
Wet jelly mass (g)	8.86	7.08	4
Dry foam			
Thickness (nm)	19.7	1.4	8
Fractional air space (ml ml^{-1})	0.998		2
Volume* (ml)	469		
Chamber volume* (ml)	155		

Larvae were Stage 22 (approximately 117–127 h old).
Data for dried foam were obtained after the larvae had escaped from the nests.
N is number of nests.
CI is confidence interval.
*Calculated from values given in this table.

on the bottom of the nest, until it reached the outside surface, whereupon the larvae commenced their escape.

Shortly before larval escape, the volume of the foamy pool averaged 48 ml, of which air bubbles accounted for 77 % (Table 1). The larval density (15.7 larvae ml^{-1}) of the foam is misleading, because the larvae were not uniformly distributed. They were able to swim freely through the wet foam, but tended to congregate at the upper and lateral edges.

Embryonic and larval development

The number of eggs laid in seven nests in which all eggs were accounted for, ranged from 501 to 1226 and averaged 854 (CI=248). Ovum diameter (measured at Stages 8–10 in four nests) was 1.705 mm (CI=0.035 mm).

We inferred age from developmental stage, because we never observed oviposition.

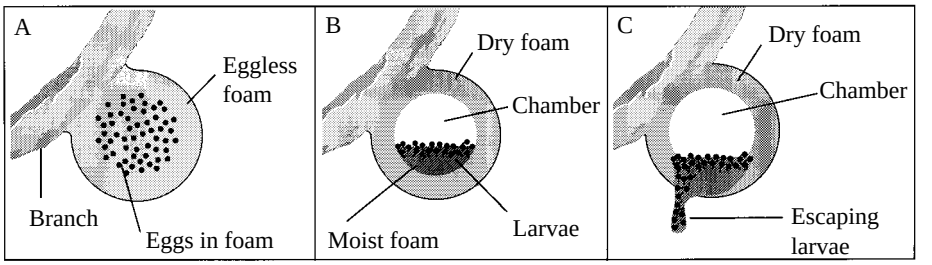

Fig. 1. Cross sections of a *Chiromantis xerampelina* nest at three stages of its life. Initially (A), the eggs are dispersed within the centre of the foam and are surrounded by a layer of eggless foam. After hatching (B), the larvae swim in a pool of moist foam, which settles at the bottom of the chamber that forms inside the shell of dry foam. The larval pool gradually descends through the outer dry shell until the larvae escape from the nest through a hole near the bottom (C).

Jennions *et al.* (1992 and personal communication) states that egg-laying in this species occurs between 20:00 and 06:30 h, and that the female almost always returns to the nest on the following night to apply eggless foam to the outside of the nest. One nest had no external eggless foam and contained eggs at Stage 8 (mid-cleavage) at 16:00 h on the day of collection. It had presumably been laid early the previous night. Three other nests, collected at the same time, all had the external foam layer and were at Stage 10 (dorsal lip), indicating that the eggs had been deposited 2 nights before collection. Thus, the accuracy of estimated fertilization time is ±6 h. These four nests were subsequently incubated at 25 °C, and their developmental stages were plotted against time. The resulting graphs were superimposed to determine the apparent difference in oviposition time. We added stage data for four other clutches that were between Stages 13 and 15 on the day of collection. The resulting graph was practically linear up to Stage 22 if corrected for the assumed times of oviposition, and the graph extrapolated to about Stage 4 (four-cell) at zero time (Fig. 2). This relationship was used to assign a '25 °C age' to staged larvae of unknown age.

At 25 °C, the embryos reached hatching Stage 22 after 83 h (CI=4 h; N=7). None hatched spontaneously at earlier stages. Hatched larvae remained in the nest at Stage 22 until they were about 132 h old (CI=15 h; N=6), when they escaped through a hole in the bottom of the nest. Given the accuracy of oviposition time, it is possible to estimate a period of 3.5 days from laying to hatching, and 5.5 days from laying to emergence from the nest.

Although the larvae remained at Stage 22 for almost 2 days after hatching, they underwent further maturation in the nest. This was evident in lengthening and arborization of the gills, progressive development of melanophores and iridophores on the back and in the eyes, and lengthening of the tail. The ratio of tail length to total body length (I) was described by the equation: I=0.405+0.00238A (r=0.94; N=15), where A is age in hours. Larvae in the nest did not gulp air, but relied on their gills and skin for gas exchange.

Two relatively small nests collected on the day following oviposition had eggs visible

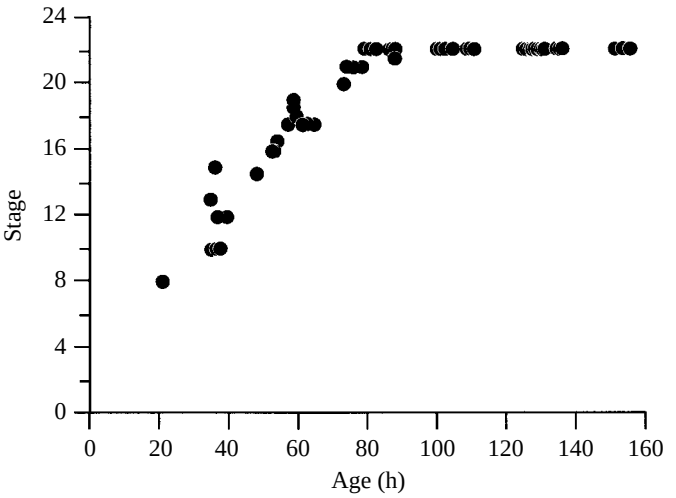

Fig. 2. The relationship between developmental stage (Gosner, 1960) and estimated age in eight nests of *Chiromantis xerampelina*, incubated at 25 °C. Each point is a mean value for 2–3 embryos or larvae. The data have been superimposed to obtain a tight fit and to indicate fertilization time. Linear regression of the data between Stages 8 and 21, inclusive, is: S=4.0+0.221A (r=0.95; N=25 points), where S is stage and A is age in hours.

on the surface. Dissection of one that was frozen showed the eggs distributed evenly throughout the foam. The other was incubated until hatching, with the result that 30 % (319/1071) of the eggs in the outer foam dried out and perished. Seven other nests, collected more than 24 h after oviposition, had a thick layer of eggless foam around the eggs, and only 5.5 % (CI=4.1 %; arcsin-transformed) of the eggs in these nests dried out. Of the mean total number of eggs laid (854), 762 (CI=260) reached hatching stage, giving an 89 % survival rate in these nests.

Escape from the nest occurred over a period of a few hours as the larvae dripped in small groups through a hole in the bottom of the nest (Fig. 1). In nests suspended from plants, the larvae dripped directly into the water below. Those escaping from nests attached to a vertical surface stuck to the surface and gradually slipped down it to the water. The last larvae to leave the nest apparently had difficulty breaking the surface tension of the foam, and a few dried larvae were sometimes found on the bottom of otherwise vacated nests.

Perivitelline space

Embryos appeared to be surrounded by two membranes less than 0.02 mm apart. This was interpreted as a thin jelly capsule bounded internally by the true vitelline membrane and externally by an outer jelly layer. The volume of the perivitelline space, including the embryo (Fig. 3), and the surface area of the vitelline membrane were calculated from measurements of its average diameter in 49 eggs from seven nests. At Stage 13 (45 h old at 25 °C), when the vitelline membrane began to lift away from the embryo, the mean volume was 2.6 µl and the surface area was 9.2 mm^2. Just before hatching, at Stage 21 (78 h

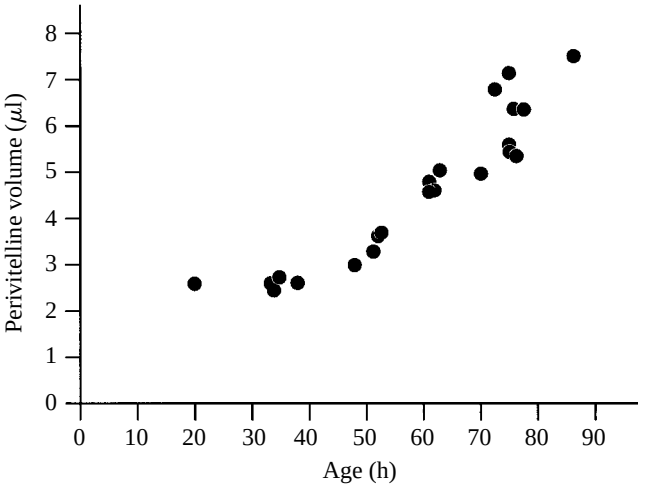

Fig. 3. The volume of the perivitelline space of *Chiromantis xerampelina* eggs during development at 25 °C.

old), the volume had increased to about 7.2 l, and the surface area to 18.1 mm^2. The embryo contacted the vitelline membrane at several locations and deformed it away from a perfect sphere, especially in later developmental stages. However, calculations of volume based on a prolate spheroid averaged only 5.4 % less than, and were always within 10 % of, the calculations based on a sphere.

Oxygen consumption and larval mass

Atmospheric P_{O_2} was about 17 kPa at 25 °C and 85 kPa barometric pressure (the conditions that prevailed at Harare). After equilibration with aerated water in the respirometers, therefore, the first measurements of oxygen consumption ($\dot{V}_{O_2}$) were made at a P_{O_2} of about 15 kPa. In hatched larvae at Stage 22 and 125–132 h old, $\dot{V}_{O_2}$ began to decline immediately with decreasing P_{O_2}, but the change was slight above 10 kPa (Fig. 4). There was no sharp 'critical' P_{O_2} in this species, but below approximately 10 kPa, $\dot{V}_{O_2}$ clearly began to decrease more steeply. $\dot{V}_{O_2}$ of unhatched embryos was essentially independent of P_{O_2} above 10 kPa, which indicates that periodic stirring of the respirometer water prevented boundary layers around the eggs from limiting oxygen uptake.

Assuming that $\dot{V}_{O_2}$ was independent of P_{O_2} above 10 kPa, we averaged the values and traced the changes in metabolic rate during development. $\dot{V}_{O_2}$ increased with development time, reaching 1.39 $\mu l\,h^{-1}$ at hatching Stage 22 (Fig. 5). A least-squares, third-order polynomial curve fit gave the equation: $\dot{V}_{O_2}=1.68-0.08614A+0.0014A^2-0.000004873A^3$, where $\dot{V}_{O_2}$ is in $\mu l\,h^{-1}$ and age (A) is in h (r=0.96; N=58). This equation fits the data well between 34 and 132 h; $\dot{V}_{O_2}$ before 34 h was assumed to increase linearly from 0 to 0.16 $\mu l\,h^{-1}$. The total oxygen consumed by an individual

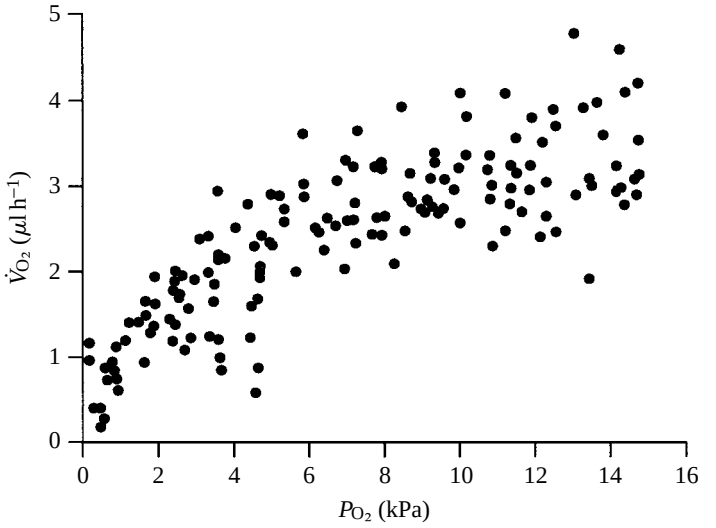

Fig. 4. Rate of oxygen consumption ($\dot{V}_{O_2}$) of Stage 22 hatched larvae of *Chiromantis xerampelina* at 25 °C in relation to ambient P_{O_2} in the respirometer water. Each point is an individual larva selected at random from eight nests.

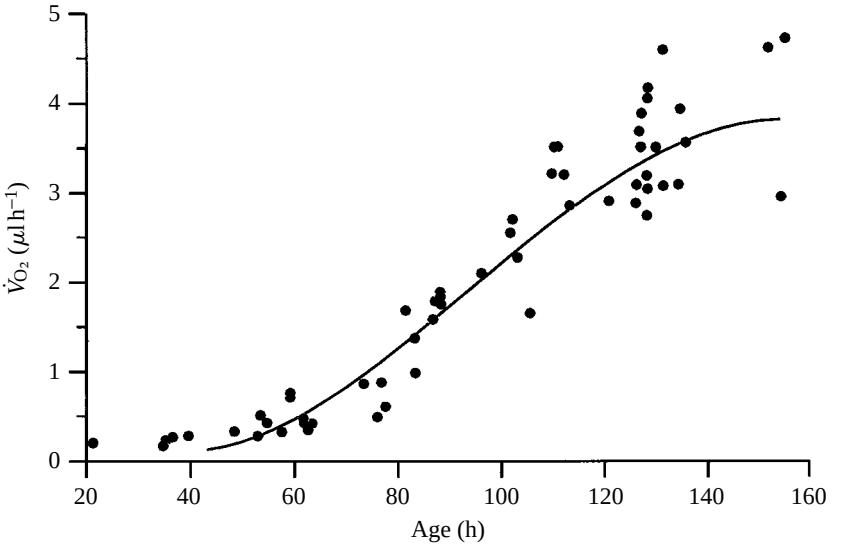

Fig. 5. Rates of oxygen consumption ($\dot{V}_{O_2}$) of embryos and larvae of *Chiromantis xermapelina* at 25 °C. Each point represents a mean value for one or two animals in the respirometry chamber. A polynomial regression line has been fitted to the data.

embryo, estimated by integrating $\dot{V}_{O_2}$, was 30 μl until hatching time at 83 h, and a further 123 μl from hatching until the escape from the nest at 132 h.

Body and gut masses of Stage 22 larvae were measured in five nests (Table 1). The

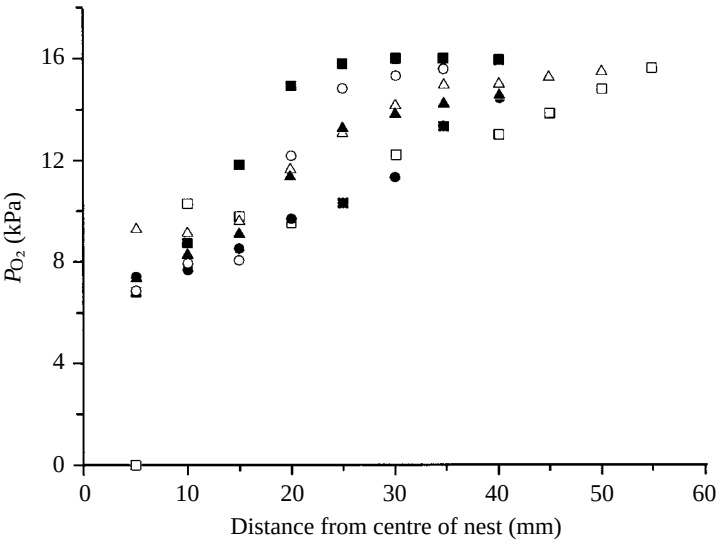

Fig. 6. Distribution of P_{O_2} in the foam through the centre of four nests of *Chiromantis xerampelina* attached to glass plates. Distance is measured perpendicularly from the glass. Two symbols (●, △) are from nests with pre-hatching embryos (Stage 20; 72–75 h old) that were evenly distributed in the moist foam; other symbols are from nests containing larvae (Stage 22; 84–128 h old). Two nests were measured twice.

wet, gut-free body mass of the larvae averaged 3.05 mg at an average age of 117 h when each embryo had consumed a total of 105 μl of oxygen. Thus the 'oxygen cost of development' at 25 °C in this species is 34 μl mg^{-1} or 232 μl mg^{-1} dry, gut-free mass.

Oxygen levels inside nests

P_{O_2} was measured in two nests (on glass) containing unhatched embryos at Stage 20, and in eight nests (five on glass and three suspended from twigs) containing hatched larvae at Stage 22. There was no consistent difference between values from nests on glass or suspended.

P_{O_2} decreased with distance into the nest from the surface (Fig. 6). The dry superficial foam appeared to be a small barrier to oxygen uptake. The mean gradient in the outermost 10 mm of dried foam in seven nests was 1.72 kPa cm^{-1} (CI=0.6 kPa cm^{-1}). Below the dry surface layer, P_{O_2} decreased more steeply, but less regularly. In some cases, P_{O_2} increased within the wet foam; this was associated with an air-pocket leading to the outside atmosphere through incomplete sealing of the foam to the glass. The means of the lowest value found anywhere in each nest were 8.20 kPa at Stage 20 and 5.76 kPa (CI=2.35 kPa) at Stage 22. In only one measurement, in one nest, did P_{O_2} approach anoxia (0.08 kPa).

The mean wet foam P_{O_2} was calculated from all values measured at locations more than 20 mm beneath the foam surface. For two nests of unhatched embryos, the mean was 10.21 kPa and for seven nests of hatched larvae, it was 9.74 kPa (CI=2.12 kPa). There was no relationship between mean wet foam P_{O_2} and embryonic or larval age. However,

Table 2. P_{O_2} *measured at selected depths in the pool of wet foam containing pre-emergence larvae (late Stage 22; >110 h old)*

Depth (mm)	P_{O_2} (kPa)	95 % CI
Surface of foam	10.3	2.3
5	4.3	1.4
10	2.3	0.7
15	2.7	1.0
20	6.1	1.1

Means and 95 % confidence intervals (CI) are given for 11–12 measurements at each depth in two nests.

linear regression for Stage 22 larvae from five nests showed decreasing mean wet foam P_{O_2} with number of living larvae (L): (P_{O_2}=14.67−0.0084L; r=−0.90). In two nests just before larval escape, the P_{O_2} was lowest in the middle of the foamy pools, indicating that oxygen moved into the pools from all directions (Table 2).

Discussion

Storage and diffusion of oxygen in the nest

We view the bubbles in the foam nests of *Chiromantis xerampelina* primarily as sources of oxygen, rather than simply as structures that facilitate diffusion. An average 624 ml nest with 77 % air contains 480 ml of saturated air. At altitudes up to 1500 m, therefore, there are more than 75 ml STPD of oxygen in the nest at laying. Until hatching at 83 h, only 25.6 ml is used by all of the embryos. Some oxygen diffusion inward from the eggless foam must occur, but it is apparent that oxygen from outside the nest is not necessary until after the embryos hatch.

Hatched larvae consume a further 105 ml of oxygen until they emerge from the nest. This exceeds the amount remaining in the bubbles, so oxygen must invade the mass through the foam. The oxygen flux is probably largely diffusive, because the foam creates a continuous barrier and the bubbles are small enough to limit convection within them. Although the foam is said to become ‘hard’ (Coe, 1974), it retains enough flexibility to prevent holes or cracks from forming as it dries and to allow it to yield to pressure differences caused by diurnal changes in temperature.

Modelling of diffusive oxygen flow enables us to verify our measurements of oxygen levels within the nest and to assess their sensitivity to hypothetical changes in nest variables. We have employed a simple numerical model of oxygen diffusion into a spherical nest of eggs. It is essentially the same analysis as used previously to examine oxygen transport through the aquatic, foamless egg masses of the Australian frog *Limnodynastes tasmaniensis* (Seymour and Roberts, 1991). Briefly, the nest is considered to be a series of nested spherical shells through which oxygen diffuses radially inwards. The rate of oxygen flow ($\dot{V}_{O_2}$) through a given shell is equal to the combined $\dot{V}_{O_2}$ of all embryos inside the shell. The P_{O_2} difference across each shell (ΔP_{O_2}) depends on $\dot{V}_{O_2}$, the

geometry of the shell (inner and outer radii, r_i and r_o) and Krogh's coefficient of oxygen diffusion through foam (K_{O_2foam}):

$$\Delta P_{O_2} = \frac{\dot{V}_{O_2}(r_o - r_i)}{K_{O_2foam} \times 4\pi r_i r_o} \quad . \tag{1}$$

This equation produces a non-linear distribution of P_{O_2} along the nest radius. K_{O_2foam} is assumed to be equal to K_{O_2jelly}, 2.9×10^{-7} cm^2 min^{-1} kPa^{-1} at 25 °C, according to Seymour and Bradford (1987), modified to account for the fraction of the foam that is filled with air (f_a):

$$K_{O_2foam} = \frac{K_{O_2jelly}}{1 - f_a} \quad . \tag{2}$$

This equation ignores the resistance of oxygen diffusion in air, because Krogh's coefficient for oxygen diffusion in air is more than 250 000 times that of water, and therefore can be considered to be infinite. The model assumes steady-state conditions which are, of course, never likely to occur in a nest that is drying out, changing shape internally and contains growing larvae. Moreover, steady-state modelling is of little use in the early nest, in which oxygen is supplied by the bubbles in the foam. It becomes increasingly valid after hatching, however, when oxygen must diffuse in through a layer of dried foam.

Initially, we incorporate our mean nest measurements into the model (Table 1). Thus, the model nest contains 695 live eggs distributed evenly in a foamy sphere, 3.33 cm in radius, surrounded by eggless foam to a radius of 5.3 cm. $\dot{V}_{O_2}$ of individual larvae is taken from Fig. 5 to be 1.4 μl h^{-1} at hatching (83 h), or 3.5 μl h^{-1} at emergence (132 h). The fraction of foam filled with air (f_a) is assumed to be 0.77 for the wet foam and 0.998 for the dry foam (Table 1). At hatching, the model predicts a radial P_{O_2} gradient in the dry foam averaging 0.55 kPa cm^{-1}, and at emergence it becomes 1.38 kPa cm^{-1}. These values are similar to the mean gradient measured with the oxygen electrode between hatching and emergence (1.72 kPa cm^{-1}). Such small gradients are possible only because the foam dries out so completely. If the outer foam retained its initial fractional air content of 0.77, the model predicts that embryonic $\dot{V}_{O_2}$ would require a P_{O_2} gradient of about 140 kPa across the foam by the time of hatching. This is clearly impossible in an atmosphere of 17 kPa P_{O_2}. In fact, the model shows that diffusion is insufficient to satisfy the oxygen demands of even early unhatched embryos, despite the increase in K_{O_2} caused by the bubbles and drying of the superficial foam.

Inside the dried foam layer, diffusion is severely restricted by water in the foam. Assuming a moist foamy compartment of 155 ml and a $\dot{V}_{O_2}$ of 1.4 μl h^{-1} in 695 hatchling larvae, the model predicts that conditions would become anoxic beneath the top 1 mm of moist foam. This explains why the larvae swim to the upper and lateral edges of the moist foam shortly after hatching. Here, they are able to position their gills in the oxygenated layer of foam, and it is likely that their swimming movements distribute oxygen into the deeper layers of the foamy pool. Interestingly, the larvae do not go to the bottom of the moist foam compartment, although P_{O_2} increases there (Table 2). The tendency for larvae to swim upwards may be a response to gravity, rather than orientation towards higher P_{O_2}.

Although significant amounts of oxygen diffuse through the dried foam after the embryos hatch, it would be erroneous to conclude that drying of the outer layer of foam is essential for adequate oxygenation of pre-emergent larvae. Irrespective of how much of the outer foam dries, the larvae could still survive by swimming to the edge of the moist foam, wherever it may be. If a partly dried nest were rained upon, and the underlying dry layer prevented larvae from reaching the relatively impermeable layer on the nest surface, enough oxygen would remain in the nest to satisfy their requirements for an estimated 20 h, during which the nest could dry out or the larvae escape. Even in the seemingly catastrophic event of a fresh nest being submerged in rising water, enough oxygen would be present in the nest to supply the embryos' requirements well beyond hatching, and recently hatched larvae could swim away from the nest and continue development in the water (R. S. Seymour and J. P. Loveridge, unpublished observations).

Respiratory role of the perivitelline space and jelly capsule

Freshly laid anuran eggs are usually surrounded by a considerable volume of jelly, which absorbs water and swells to form a rather thick capsule over the vitelline membrane (Beattie, 1980). The capsule impedes oxygen diffusion, potentially causing severe hypoxia in later development, when embryonic oxygen demand increases. However, the embryo causes water to be absorbed osmotically into the perivitelline space beneath the vitelline membrane (Salthe, 1965). This increases the oxygen conductance of the capsule by increasing its surface area and decreasing its thickness. These changes in oxygen conductance are essential for adequate embryonic oxygenation in the terrestrial eggs of *Pseudophryne bibronii* (Seymour and Bradford, 1987) and the gelatinous aquatic eggs of *Limnodynastes tasmaniensis* (Seymour and Roberts, 1991). These species have eggs with relatively thick capsule jelly (0.5–3 mm), and P_{O_2} differences of 3–5 kPa prevail across their capsules. *C. xerampelina*, in contrast, produces eggs with capsules less than 0.02 mm thick, and the P_{O_2} gradient is correspondingly small. A model of oxygen diffusion through jelly of a single egg (Seymour and Bradford, 1987), applied to *C. xerampelina*, predicts a P_{O_2} difference of 0.35 kPa at Stage 13, when the vitelline membrane begins to lift away from the embryo, increasing to 0.52 kPa at pre-hatching Stage 20, when the perivitelline volume has more than doubled (Fig. 3). These P_{O_2} differences are quite small, despite high rates of oxygen consumption. It is clear that the increase in perivitelline volume has little respiratory significance in *C. xerampelina*. Water absorption may be important to provide a controlled environment for the embryo, or to allow space for embryonic growth, or it may be simply a vestige from ancestors that deposited gelatinous eggs within thick capsules.

The thin capsules of *C. xerampelina* clarify the respiratory role of thicker capsules in species that do not construct foam nests. In most aquatically breeding frogs, the capsule separates the embryos from one another, distributing them evenly within the egg mass and preventing aggregations in which competition for oxygen would be severe (Seymour and Roberts, 1991). In the foam-nester, however, the bubbles perform this function, and consequently a thick capsule is not necessary. Thin capsules may be a general characteristic of foam-nesting species, occurring, for example, in the distantly related families Rhacophoridae (*Chiromantis rufescens*; Coe, 1974), Hylidae (*Hyla rizibilis*;

Haddad *et al.* 1990) and Myobatrachidae (*Limnodynastes dorsalis*; R. S. Seymour and J. D. Roberts, unpublished observations).

Delayed emergence

The extended period in which the larvae remain in the nest at Stage 22 has several advantages. Although freshly hatched larvae can survive in free water, they avoid predation for longer and mature further in the nest, becoming stronger swimmers. It is also possible that the delay permits all of the larvae to reach sufficient maturity to emerge from the nest simultaneously. Because temperature differences profoundly influence rates of development in this species (R. S. Seymour, unpublished observations), the larvae at different locations in the nest may hatch at different times. The time between hatching and emergence could permit the slower-developing larvae to 'catch up' with the faster ones, as has been suggested to occur in clutches of ratite birds (Hoyt *et al.* 1978) and some reptiles (Thompson, 1989). One advantage of simultaneous emergence in foam-nesting frogs is that it avoids the danger of desiccation of larvae that might otherwise occur if they emerged in small groups over an extended period. We observed that the last larvae to leave the nest often died because they could not individually break free from the surface tension of the foam. Other larvae, emerging from nests on vertical surfaces, usually wriggled down the surface together. Those that became separated from the main stream often stuck to the surface and perished.

The stimulus and the mechanism of emergence from the nest are unclear in *C. xerampelina*. Although Coe (1974) suggests that the larvae of *C. rufescens* leave the nest only when heavy rains dissolve it, larvae of *C. xerampelina* spontaneously emerge from dry nests, about 2 days after they hatch. Falling energy reserves do not stimulate emergence, as the larvae have plentiful yolk remaining (Table 1) and can survive without feeding for longer than a week after emergence (R. S. Seymour, unpublished observations). It is possible that emergence is related to decreasing levels of available oxygen. Low P_{O_2} is known to stimulate hatching and liberation of amphibian larvae from the clutch (Petranka *et al.* 1982; Bradford and Seymour, 1988). Some locations in the foamy pool become anoxic (Fig. 6), and most of the moist foam becomes hypoxic (Table 2). $\dot{V}_{O_2}$ is limited by low P_{O_2} (Fig. 4) and becomes progressively more limited as the volume of the moist foam diminishes, as the larvae become more crowded and as their oxygen demands increase. The larvae may eventually reach a situation in which the struggle to obtain sufficient oxygen becomes intolerable and they have to leave the nest.

Limits to nest size

Incorporation of bubbles into foam nests eliminates the limits to the size of egg masses set by the constraint of the rate of oxygen diffusion through jelly. Solid, gelatinous egg masses can be only a few millimetres thick or contain few embryos with small oxygen requirements (Seymour and Roberts, 1991). The nest model above predicts that a spherical *C. xerampelina* egg mass with an egg density of (695/624=1.1 eggs ml^{-1}; Table 1) could be only 2.3 cm in diameter and contain only seven eggs respiring at 1.4 $\mu l\,h^{-1}$. Actual clutches are a 100 times larger than this.

While foam-nesting obviously increases the potential number of larvae that can be

produced on land, it is interesting to consider whether oxygen availability places any limit on this number. For embryos up to hatching, this is apparently not the case. Each embryo has its own supply of oxygen in the bubbles surrounding it. Thus, the number of embryos and the oxygen supply are both directly related to the volume of the nest. After hatching, the larvae compete for oxygen at the interface of moist and dry foam, and the number of larvae could theoretically become limited by the surface area of the interface. Even in the most densely populated nests, however, no larvae were asphyxiated and a large interface remained unoccupied at the bottom of the nests. It is most likely that clutch size is limited, not by respiratory constraints on the larvae, but simply by the size of the female. There are limits not only on the number of eggs she can carry (Salthe and Duellman, 1973) but also on the amount of foam she can produce. Careful observations of the breeding behaviour of *C. xerampelina* (Jennions *et al.* 1992) reveal that the female interrupts foam production two or three times on the first night of nest construction and re-enters the pool during each break, presumably to absorb enough water to continue making foam.

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