GAS EXCHANGE, METABOLISM, AND "VENTILATION" IN GELATINOUS FROG EGG MASSES[1]

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Earlier studies of gelatinous egg masses laid by many amphibians suggested that the egg mass center was extremely hypoxic and that embryos located there suffered delayed growth and high mortality. The present study has reassessed conditions for respiration within the spherical egg masses of the pickerel frog, *Rana palustris*. Partial pressures of O_2 and CO_2, and the pH of egg jelly, as well as lactate concentration of embryonic tissue, were measured at 1-cm depth increments from surface to center of the egg mass. Oxygen uptake of intact egg masses and eggs separated from the mass was determined as a function of temperature and ambient O_2 levels. Although significant gradients for O_2, CO_2, and pH existed from surface to center, the magnitude of these gradients was too small to directly affect O_2 uptake of the egg mass or individual eggs under a variety of experimental conditions likely to occur in nature. Exchange of gases between the egg mass surface and center occurs primarily by diffusion through the egg jelly, which has a Krogh's O_2 diffusion constant 75% that of distilled water. However, intense convection of fluid within each egg, generated by the embryo's ciliated epithelium, functionally reduces by 17% the diffusion distance between egg mass surface and center by generating a convective "ventilation." These data collectively refute earlier findings by demonstrating that the center of ranid egg masses is not severely hypoxic and not limiting to aerobic metabolism.

INTRODUCTION

Investigation of how respiratory processes change during amphibian metamorphosis has revealed much about "amphibious" gas exchange in vertebrates generally. With few exceptions, however, these physiological experiments on amphibia have focused on late larval development and the functional replacement of gills by lungs (see Burggren [1984] for review). Very little is known about the respiratory physiology of amphibian embryos and of gas exchange within the egg, in spite of intense interest in embryonic respiration in other vertebrates (see Seymour 1984).

The study of amphibian embryos presents difficult practical problems but also fascinating biological ones. Amphibian embryos typically weigh only 5–25 mg at hatching, and this minute size precludes many forms of physiological experimentation. Consequently, most studies of respiratory processes in amphibian embryos have consisted of measurements of metabolic rate, often on artificially fertilized eggs stripped of their surrounding jelly (Bialaszewicz and Bledowski 1915; Parnas and Kraskinska 1921; Brachet 1934; Savage 1935; Wills 1936; Detwiler and Copenhaver 1940; Barth 1946; Gregg 1962; Rose, Armentrout, and Roper 1971; Weigman and Altig 1975; Adolph 1979). The very small size of many amphibian embryos suggests that the exchange of respiratory gases between environment and embryonic tissues potentially could be met mainly or even entirely by diffusive rather than convective processes. However, many amphibians may lay up to several thousand eggs in gelatinous aggregations as large as 10–20 cm in diameter (DeGraaf and Rudis 1983). Although embryos may move within their own eggs, the position of any individual egg is fixed within the egg mass from time of laying until hatching. The physiological conditions for gas exchange within such large egg masses have been

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measured in only a few instances, though the early literature is strongly of the opinion that the egg mass interior must be quite hypoxic (Savage 1935; Barth 1946; Gregg 1962). For example, Savage (1935) reported that the partial pressures of oxygen in the center of egg masses of *Rana temporaria* may be as low as 4 mmHg, with pH as much as 0.8 units lower than ambient water. Considerable anaerobic metabolism is indicated by Barth's (1946) observation that lactic acid concentrations in the eggs of *Rana pipiens* in the center of the egg mass are nearly 200 times greater than in individual eggs previously separated from the egg mass. The accuracy of these early observations could potentially be verified if data were available for rates of embryonic respiration and the gas diffusion properties of egg mass jelly. Unfortunately, the physical properties of the amphibian egg mass with respect to diffusion of respiratory gases are completely unknown.

Amphibian embryos have considerable tolerance to hypoxia and even anoxia (see Burggren [1984] for references). If, however, the center of a ranid egg mass is indeed severely hypoxic for much of development, as indicated by the early literature, then it might be anticipated that survival and development rate might be lower in embryos in the center of egg masses. This is apparently not the case, for our unpublished field observations on undisturbed, large egg masses of ranid species in Massachusetts indicate that embryos from the surface and center of the mass hatch at the same developmental stage, at the same time, and with the same success.

Another complicating factor in the analysis of gas exchange involves symbiotic relationships between amphibian eggs and the often considerable algal populations living not only in the jelly matrix but also actually within the egg (Orr 1888; Gilbert 1942; Hutchison and Hammen 1958; Hammen and Hutchison 1962; Hutchison 1971; Gatz 1973). Eggs of the salamander *Ambystoma maculatum* that are inhabited by *Chlamydomonas = (Oophila) amblystomatis* have a lower mortality rate, faster growth rate, and earlier hatching time than noninhabited eggs. Although it is thought that this improved survivability results from the release of an unidentified growth-promoting factor (Hammen and Hutchison 1962), it is possible that photosynthesis in eggs with highly concentrated algal populations might significantly raise O_2 levels and reduce CO_2 levels within the egg mass (Moore, Keammerer, and Smith 1974). This assumes, however, that the interior of the egg is hypoxic and hypercarbic in the absence of photosynthesizing algae, a condition which to my knowledge has not been demonstrated experimentally.

The intent of the present study was thus to investigate respiration in embryos of the pickerel frog *Rana palustris*, which are laid and develop in large egg masses. These embryos were treated not as free-living entities as in most previous studies, but rather were studied in the context of subunits of a larger physiological unit—the gelatinous egg mass.

MATERIAL AND METHODS

All experiments were performed on egg masses or individual eggs of the pickerel frog, *Rana palustris*. Egg masses were collected during late April, 1984, from a 0.1-ha permanent pond on the edge of the Quabbin Reservoir in Hampshire County, Massachusetts. Egg masses were transferred intact to the laboratory, where they were maintained in pond water at 5–7 C for a maximum of 2 wk before study. All experiments were performed on embryos at developmental stages 17–21 (Gosner 1960). Hatching in *Rana palustris* occurs at stage 21.

1. MEASUREMENTS WITHIN THE EGG MASS

In the first experimental series, egg masses were maintained under conditions of constant, subdued light (30–35 ergs · cm^{-2} · s, fluorescent bulbs) in a thermostated bath containing pond water. After the egg masses had remained undisturbed for a period of 6 h, O_2 and CO_2 partial pressures and pH of the jelly matrix surrounding the eggs were measured at the surface of the roughly spherical egg mass and then at depths increasing in 10-mm increments to its center. Measurements were made at 5 C with egg masses in air-saturated water and at 15 C with egg

masses in mildly hypoxic (P_{O_2} = 85 mmHg), normoxic (150 mmHg), and mildly hyperoxic (185 mmHg) water. Water partial pressures were altered by gently flowing the appropriate gas mixture from a Wösthoff gas-mixing pump through an air stone placed in the water bath containing the egg masses.

Oxygen and CO_2 partial pressures at various depths within the egg masses were measured with the electrodes and electrometer of an Instrumentation Laboratory μ13 blood-gas system. The electrodes, which in factory configuration measure partial pressures on static samples injected into a sample chamber, were removed from their thermostated cuvettes and suspended in a vertical position from micromanipulators positioned immediately over the egg masses in the water bath. The electrode tip and major portion of the electrode body remained in the water bath at all times but for calibration (see below), thus ensuring constant electrode temperature. By adjustment of the micromanipulators, the tips of the electrodes could be inserted at controlled depths into an egg mass. Great care was taken to ensure that no gas bubbles or water was introduced into the egg mass along the track of electrode movement. This was relatively easy to achieve since the egg jelly invariably closed very tightly around the electrode as it penetrated the egg mass. Consequently, an individual egg mass could be repeatedly penetrated by the electrodes without any apparent disruption of the egg mass shape or change in position of individual eggs.

Initially, extensive control experiments were performed in which the tips of the electrodes were lowered to various depths in water of known and adjustable gas composition, and gas partial pressures were constantly monitored. These experiments revealed that partial pressure measurements were extremely stable even when the electrodes were constantly repositioned in the water column. Importantly, intentional mechanical disturbance of the membrane over the electrode tip, which could be anticipated as the electrodes were positioned at various depths within the jelly of the egg masses, resulted in only a transient disturbance in the electrode output voltage. This spurious signal was immediately eliminated when movement of the electrode stopped, and the electrometer reading invariably returned to the same partial pressure reading prior to electrode repositioning.

Another concern involved potential artifacts induced by the O_2 consumption of the O_2 electrode and the CO_2 capacitance of the electrolyte in the CO_2 electrode. In both cases this could induce signal drift if O_2 or CO_2 depletion of the unstirred jelly mass occurred in the immediate vicinity of the electrode tips. In fact, at all measurement temperatures the O_2 and CO_2 signals stabilized within 1 (O_2)–4 (CO_2) min of moving the electrode tips to a new position within the jelly. Signal drift indicating changes in gas partial pressures in the vicinity of the electrodes because of their O_2 or CO_2 "consumption," did not begin for approximately 4 (O_2)–8 (CO_2) min.

The pH of the jelly in the egg mass was measured at the same increments of increasing depth with a Fisher Microprobe combination pH electrode. The accuracy of this pH measurement system in the egg mass was also verified by the same control experiments described above for the respiratory gas measurements.

The O_2 and CO_2 electrodes were calibrated at the experimental temperature with appropriate gas mixtures between each insertion into a new egg mass. Calibration was performed while the electrodes were still partially submerged in the water bath to ensure temperature stability. This was achieved by covering the submerged electrode tip with a glass cylinder through which humidified calibration gases flowed. Similarly, the pH electrode was calibrated before each insertion with the appropriate buffers maintained at the water bath temperature.

After P_{O_2}, P_{CO_2}, and pH gradients from the outside to the center of the egg masses were determined, individual eggs were quickly dissected from various depth levels in each egg mass, the egg broken open, and the embryo extracted from within. The embryos were then immediately homogenized in 8% perchloric acid. The homogenate was centrifuged and analyzed for lactate as described by Quinn and Burggren (1983). Lactate concentrations

of embryonic tissue were expressed as mg lactate · 100 ml^{-1} (mg %) of embryonic tissue.

2. OXYGEN DIFFUSION PROPERTIES OF EGG JELLY

A modification of the technique of Ultsch and Gros (1979) was used to determine Krogh's diffusion constant for oxygen in water and egg mass jelly. Essentially, the apparatus consisted of three cylindrical chambers (diameter 15 cm) arranged end to end. The central chamber was 2.5 mm long and constructed of glass. It was separated from the other two chambers (100 mm long and constructed of plastic) by a 150-μm thick silicone membrane over each end. The central chamber was filled with either water or jelly through a small filling port, which was then sealed. In essence, then, the two end chambers were separated by a layer of membrane-enclosed jelly or water through which O_2 diffused. One end chamber was filled with 100% O_2. The other chamber was filled with 100% N_2 and in addition contained an Instrumentation Laboratory O_2 electrode. The entire apparatus was thermostated to 20 C.

Initially, 100% N_2 was passed through the end chamber containing the O_2 electrode, while 100% O_2 was passed through the other end chamber. These gas flows were maintained for 1 h after assembling the apparatus with jelly or water in the central chamber. Then, O_2 flow was maintained, while the N_2 flow was stopped. Oxygen diffusing through the center chamber caused a rise in the P_{O_2} of the chamber initially containing 100% nitrogen. The diffusion coefficient for O_2 through the substance separating the end chambers could then be calculated from the rate of increase of O_2 in the N_2-filled chamber, using the instantaneous P_{O_2} gradient among end chambers, chamber volumes, central chamber thickness (i.e., diffusion path length) and cross-sectional area, and the silicone membrane O_2 diffusion properties (Gros and Moll 1971). To test for diffusion of O_2 into the N_2-filled chamber directly through the walls of the chambers rather than through the fluid in the central chamber, control experiments were performed with aluminum foil rather than silicone membranes to separate the center from end chambers. In fact, a constant and reproducible diffusion of O_2 into the N_2-filled chamber occurred at a slow rate. This rate of diffusion was subtracted from that measured when silicone membranes were used to correct for diffusion of O_2 into the apparatus directly through the chamber walls.

3. RESPIROMETRY OF EGG MASSES

Individual egg masses were carefully transferred to translucent plastic chambers containing a known volume of aerated pond water. The total volume of the respirometers ranged from 3,882 to 3,899 ml. The volume of each egg mass was estimated by assuming the mass to be spherical and calculating its volume based on diameter. The total volume of water in the respirometer from which the embryos consumed O_2 was thus determined by subtracting egg mass volume from total respirometer volume.

The chambers sat in a thermostated water bath to regulate egg mass temperature. Temperature was monitored in the center of the egg mass with a Cole Parmer electronic thermometer and probe. Six hours after egg masses were transferred, the gastight lids were secured without introducing gas bubbles into the water in the respirometer. A water sample was then taken into a syringe via a stopcock fitted in the respirometer lid and analyzed for P_{O_2} in an Instrumentation Laboratory μ13 blood-gas analyzer. At frequent and regular intervals thereafter water was repeatedly drawn into a syringe and then expelled back into the respirometer without introducing bubbles into the water. This served to stir up the water surrounding the egg mass and prevent stagnation immediately next to the edge of the mass. Immediately after the stirring periods, additional water samples were taken and their P_{O_2} determined. With known values of the P_{O_2} of respirometer water from successive samples, the time interval between samples, the O_2 capacitance of the water at the experimental temperature, and the volume of the water in the respirometer, the O_2 consumption of the egg mass could be determined.

After O_2 consumption of the intact mass was measured, each egg mass was carefully pushed through a screen with 7-mm square

mesh, effectively separating each egg from the mass and removing surrounding jelly without damaging the egg capsule itself. At this time the number of embryos that had been in each mass was counted. The separated eggs were returned to the respirometers, allowed to acclimate for 4 h, and their O_2 uptake measured as described above for intact masses. After these measurements, streptomycin (160 $\mu g \cdot ml^{-1}$), penicillin (100 $\mu g \cdot ml^{-1}$), and tetracycline (25 $\mu g \cdot ml^{-1}$) were added to the water in the respirometer to minimize microbial respiration, and the O_2 consumption of the separated eggs was measured a final time.

From the mean mass of 10 embryos and the total number of embryos in each egg mass, the total mass of embryonic tissue in each egg mass was calculated. Oxygen consumption was expressed as $\mu M\ O_2 \cdot g$ embryonic tissue$^{-1} \cdot h^{-1}$. Oxygen consumption was measured as a function of egg mass temperature and ambient water P_{O_2}, as described below.

4. FLUID CONVECTION WITHIN EGGS

Examination of embryos within their eggs under 60× magnification revealed that the ciliated epithelium of the embryo generated a rapid convective flow of fluid within the egg. The velocity of this current was quantified under a variety of conditions in individual embryos in their eggs by placing them in watch glasses under a dissecting microscope. The fluid in the egg contained suspended cellular debris that served very conveniently as markers in the fluid currents. From the time taken for a marker to pass two reference points on the embryo separated by a known distance (determined by an ocular micrometer in the eyepiece), the current velocity could be estimated. In some experiments, a microelectrode was used to inject carmine particles into the egg, which improved visualization of current patterns.

Velocities were estimated as a function of egg temperature, P_{O_2} and P_{CO_2} of the ambient water, and developmental stages.

5. STATISTICAL ANALYSES

All data were expressed as mean ± 1 SD. Treatment effects were assessed with analysis of variance (ANOVA), while differences between individual means were tested with Student's *t*-test for independent means.

TABLE 1

WATER AND SHADE AIR TEMPERATURES AT THE COLLECTING SITE

Distance from Water Surface (cm)	Temperature (°C)
2 (shaded air)	16.8
0 (water surface)	13.0
1 (under water)	12.7
2 (under water)	11.9
3 (under water)	11.5
4 (under water)	11.1
5 (under water)	10.9
6 (under water)	10.5
25 (under water)	10.0

NOTE.—Data were collected at 12:00 EST on a cloudless day at a site exposed to full sunlight.

RESULTS

1. FIELD OBSERVATIONS

Egg masses found floating at the water surface or attached to submergent plants were collected from the margin of the pond. In their positions as laid, the egg masses and the water surrounding them were exposed to direct sunlight for approximately 9 h daily. Water and shade air temperatures at noon EST were measured at the pond surface and at various depths (table 1). Only a very slight thermocline was evident, with the top centimeter or two of water being 1–2 degrees warmer than the remainder of the water column. Temperature at the center of 10 egg masses was also measured as they floated undisturbed in their natural positions. Temperature at the center of the egg masses averaged 16.5 ± 1.6 C. Notably, the center of one egg mass was 19.1 C, which was 6 degrees warmer than the temperature of the surrounding water and more than 2 degrees above that of the air over the water.

Samples of pond water that had been collected anaerobically at a depth of 4 cm, put on ice, and then analyzed in the laboratory within 2 h had a P_{O_2} of 163 mmHg (air saturation = 154 mmHg), a P_{CO_2} of 0.2 mmHg, and a pH of 5.87.

STRUCTURE AND ORGANIZATION OF THE EGG MASSES

Rana palustris is reported to lay egg masses containing from 2,000 to 3,000 eggs (Wright 1914). The spherical egg masses of *R. palustris* examined in the present study contained a mean of 959 ± 153 eggs and had a mean diameter of 89 ± 7 mm (n = 8). Ten individual eggs containing embryos of developmental stages 18–20 weighed 96.9 ± 23.9 mg and had a diameter of 5.0 ± 1.0 mm, while these 10 embryos dissected away from the egg capsule weighed 10.2 ± 4.4 mg.

3. RESPIRATORY CONDITIONS INSIDE THE EGG MASS

Gradients of P_{O_2}, P_{CO_2}, pH, and lactate concentration from the outer surface to center were measured in eight egg masses maintained in subdued light under varying conditions of temperature and ambient oxygenation (fig. 1). Significant changes in P_{O_2} with changes in depth in the egg masses occurred under all conditions, though there was considerable variation between individual egg masses, particularly at 15 C. At 5 C the P_{O_2} at the center of the egg mass was approximately 7 mmHg lower than the P_{O_2} in jelly at the edge of the mass. At 15 C and all conditions of ambient oxygen, this gradient increased to 20–35 mmHg. In one egg mass under hyperoxic conditions, the P_{O_2} gradient from egg mass surface to center was approximately 120 mmHg, but in other egg masses the P_{O_2} gradient was less than 6–8 mmHg.

A significant P_{CO_2} gradient also existed from edge to center of the egg mass (fig. 1). The mean P_{CO_2} in all conditions was about 0.1 mmHg higher at the egg mass center, which was just within resolution of the P_{CO_2} measuring system. In the single egg mass described above in which there was an unusually large P_{O_2} gradient of 120 mmHg, the attendant P_{CO_2} gradient

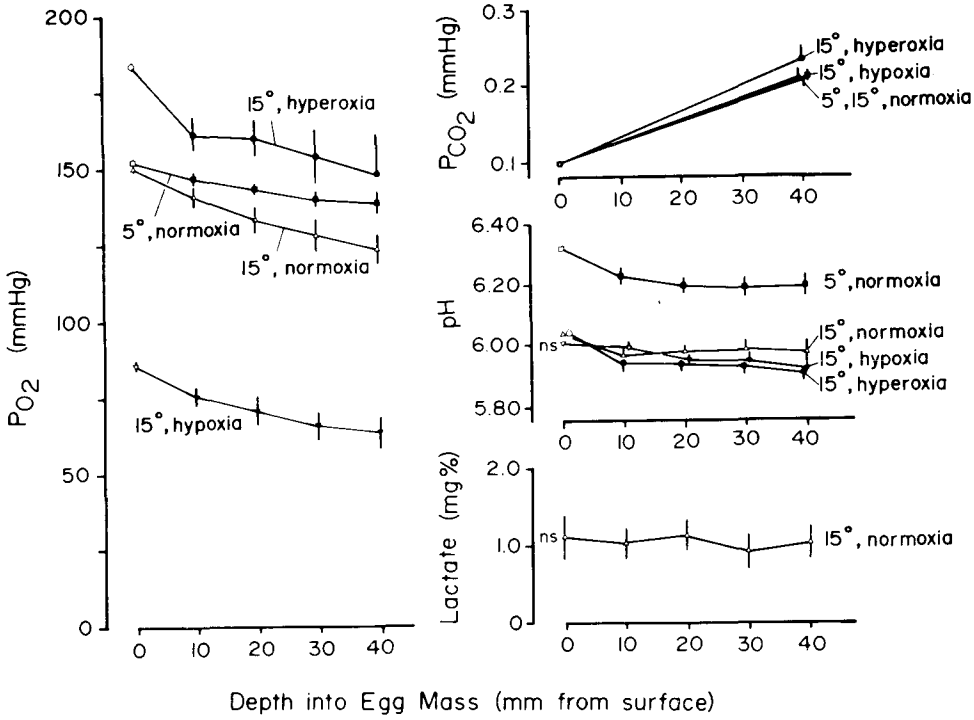

FIG. 1.—P_{O_2}, P_{CO_2}, pH, and lactate concentration at different temperatures and ambient oxygen levels as a function of depth into the intact egg mass of the frog *Rana palustris*. Mean values ± 1 SD for eight egg masses are presented. With the exception of the lines marked not significant (*ns*), all variables changed significantly as a function of depth (ANOVA, $P < .05$ or smaller). Values significantly different from those measured at the surface (open symbols) are indicated by solid symbols (independent t-test). See text for details.

TABLE 2

VALUES OF KROGH'S DIFFUSION CONSTANT ($cm^2 \cdot min^{-1} \cdot atm^{-1} \times 10^5$) FOR O_2 IN VARIOUS BIOLOGICAL SUBSTANCES AT 20 C

Medium	K	References
Water	4.25	Grote 1967
Water	3.6	Ultsch and Gros 1979
Water	3.15 ± .42	Present study
Egg jelly (*Rana palustris*)	2.38 ± .42	Present study
15% gelatin	2.8	Krogh 1919
9% serum albumin	2.6	Kreuzer 1950
Frog skeletal muscle	1.4	Krogh 1919
Frog connective tissue	1.15	Krogh 1919
Rat myocardium	1.9	Grote and Thews 1962
Human erythrocytes	1.3	Grote and Thews 1962

from edge to center was also elevated to approximately 0.5 mmHg.

The pH of the egg mass jelly was significantly lower in the center in all conditions but 15 C normoxia. Even in those conditions where significant changes occurred, the differences were only 0.05–0.01 pH units. The jelly pH at 5 C was approximately 0.25 units higher than at 15 C, which compares with a change of approximately 0.16 pH units when pure water is cooled from 15 C down to 5 C.

The lactate concentration of the embryonic tissue was about 1 mg% and showed no significant changes with increasing depth of the embryo in the egg mass (fig. 1).

4. OXYGEN DIFFUSION PROPERTIES OF JELLY

Krogh's diffusion constant for O_2 through water ($cm^2 \cdot min^{-1} \cdot atm^{-1}$) was calculated as the volume of gas (in ml at STPD) diffusing per unit time (min) per unit area (cm^2) per unit thickness (cm) when the partial pressure driving diffusion is 1 atm in the direction of gas flow (Bartels 1971). Values at 20 C for distilled water, egg mass jelly, and other media all at 20 C are presented in table 2. Krogh's diffusion constant for O_2 through jelly taken from the egg mass was 75% of that for O_2 through distilled water but still much higher than that in vertebrate tissues.

5. AEROBIC METABOLISM

Embryonic O_2 consumption ($\dot{M}O_2$) measured in seven intact egg masses (embryos of Gosner stages 19–21) was approximately 1 $\mu M \cdot g^{-1} \cdot h^{-1}$ at 7 C, increasing to approximately five times this level at 27 C (fig. 2). The Q_{10} for oxygen uptake between 7 and 17 C was 2.84, and between 17 and 27 C was 2.04.

Oxygen consumption at 17 C was measured as a function of ambient water P_{O_2} in a separate series of experiments on seven intact egg masses. Respiration by the embryos in the closed respirometers was used to reduce the P_{O_2} of the ambient water. At air saturation levels of approximately 150 mmHg, $\dot{M}O_2$ was not significantly different from that measured in the

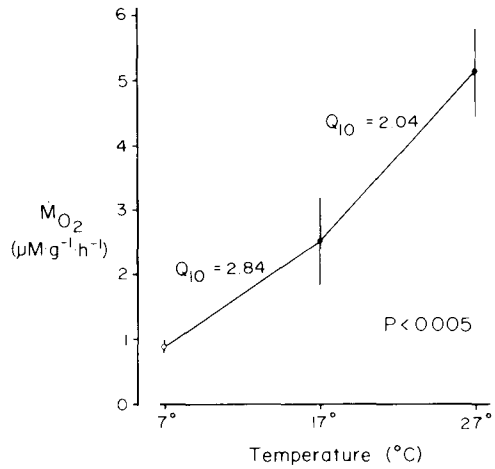

FIG. 2.—Oxygen uptake (mean ± 1 SD) as a function of temperature for seven intact egg masses. Probability level is for ANOVA of treatment effects. Values significantly different from those at 7 C are represented by solid symbols, as in fig. 1. Calculations of the temperature quotient for metabolism, Q_{10}, are given for the two 10 C temperature ranges.

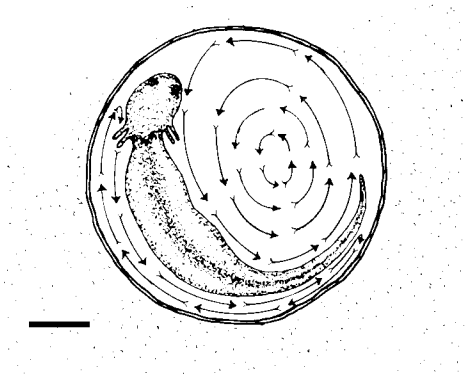

FIG. 3.—A diagrammatic representation of the egg of *Rana palustris*, showing the embryo (Gosner stage 21) and the currents in the egg fluid that the ciliated epithelium of the embryo generates. Scale bar = 1 mm.

previous experimental series at 17 C, as presented in figure 2. In this second series there was no significant effect ($P > .1$, $n = 44$) of ambient P_{O_2} on $\dot{M}_{O_2}$ from 150 mmHg down to 50 mmHg.

In a third experimental series on seven egg masses, $\dot{M}_{O_2}$ under conditions of air saturation was measured in the intact egg mass, after dissection of the individual eggs from the mass and again after addition of antimicrobial agents to the respirometer water. Oxygen consumption by these eggs was not significantly affected by removal from the egg mass or treatment with antibiotics (ANOVA, $P > .1$).

6. CONVECTION WITHIN THE EGG CAPSULE

Complex and predictable patterns of convection of fluid within the egg capsule were generated by the ciliated epithelium of the embryos (fig. 3). Although the pattern of convection was very consistent between stage 17 (the youngest embryos examined) and stage 21 (the time of hatching and subsequent loss of epithelial cilia), the actual velocity of the currents measured against the embryo's body surface varied significantly with temperature and developmental stage. At 5 C, current velocity in stage 20 embryos averaged about 0.1 $mm \cdot s^{-1}$, increasing to nearly 0.3 $mm \cdot s^{-1}$ at 25 C (fig. 4*A*). The Q_{10} for current velocity was 2.52 from 5 to 15 C, and 1.31 from 15 to 25 C.

In virtually every one of 20 embryos examined, current velocity within the egg decreased from stage 17 to 18, increased again to a high at stage 20, and then decreased again before hatching at stage 21 (fig. 4*B*).

Sporadic body movements by embryos began in the last 24–48 h of development before hatching. Even when movement resulted in total repositioning of the embryo within its egg, fluid current patterns, as indicated in figure 3, were immediately reestablished.

DISCUSSION

COMMENTS ON FIELD OBSERVATIONS

The preliminary field observations of pond water characteristics, though admittedly incomplete with respect to hourly and daily variation, indicate a very low P_{CO_2} combined with a P_{O_2} significantly higher than air saturation during midday. This condition is the probable consequence

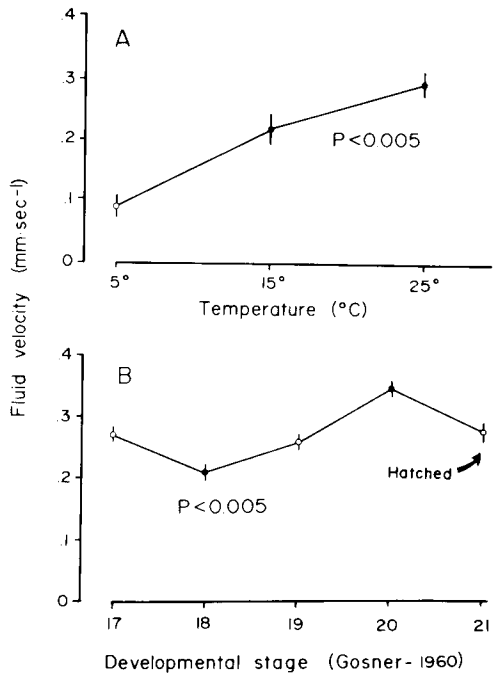

FIG. 4.—Fluid velocity against the body wall of embryos in intact eggs. Mean values ± 1 SD ($n = 20$) are presented. *A*, Fluid velocity as a function of temperature is given for stage 18 embryos. *B*, Fluid velocity at 15 C as a function of developmental stage is shown. Statistical conventions are as in fig. 1.

of photosynthetic activity by the considerable population of submergent, rooted plants in the study pond. Environmental O_2 partial pressures at or even above air saturation are clearly advantageous for animals such as the embryos of *Rana palustris,* which consume significant amounts of O_2 but are unable to move away from a hypoxic microhabitat. However, aquatic habitats can become quite hypoxic at night when photosynthesis is supplanted by respiration (Jones 1961; Garey and Rahn 1970). Though the extent of nightly O_2 depletion in the study pond was not determined, the well-documented ability of amphibian embryos to withstand severe hypoxia (Detwiler and Copenhaver 1940; Gregg 1962; Rose et al. 1971; Weigman and Altig 1975; Adolph 1979) may be an important part of the physiological repertoire of these animals.

Given that temperatures within egg masses of *Rana palustris* may rise to several degrees above that of ambient air or water at midday, it is likely that some significant elevation of egg mass temperature may persist for several hours each day. In *Rana sylvatica* there is a strong correlation between the degree of elevation of egg mass temperature above ambient and both the extent of cloud cover and the aggregation of multiple egg masses (Seale 1982). This suggests that physical factors such as infrared absorption (aided by the effect of the black color of the embryos) and possible insulating effects in large aggregation of egg masses may play a role in elevation above ambient of egg mass temperature in ranids. Elevated egg mass temperature will accelerate metabolism, development, and hatching of the embryos (Seale 1982; Petranka, Just, and Crawford 1983; also fig. 2).

GAS EXCHANGE IN THE EGG MASS

Oxygen levels are higher and carbon dioxide levels lower in outer regions compared with inner regions of the spherical egg masses laid by *Rana palustris.* With respect to oxygen, this regional heterogeneity is greater at 15 C than at 5 C and appears independent of moderate changes in ambient P_{O_2}. These observations are completely consistent with a mild O_2 depletion within the egg mass resulting from significant aerobic metabolism of the embryos. Though amphibian embryos in very early developmental stages have an extremely small O_2 consumption, typically O_2 consumption increases rapidly after folding of the neural crest (see Burggren [1984]; Bradford and Seymour [1985] for references). In fact, the mass-specific metabolic rate measured for stage 19–21 embryos in the intact egg mass of *Rana palustris* is 1.5–3 times greater than that measured in posthatch ranid larvae at similar temperatures (Burggren, Feder, and Pinder 1983; Feder, unpublished), which is appropriate for the much smaller embryos.

In spite of measured respiratory gas heterogeneity within the egg mass, the conditions for aerobic respiration at the center of the egg mass are *physiologically* very similar to those at the outer surface. In only a few instances was O_2 partial pressure at the center more than 40 mmHg lower than at the egg mass edge. This held for egg masses not only when in normoxic water but also when egg masses are exposed to mild hypoxia or mild hyperoxia (fig. 1), conditions which might occur at dawn and dusk, respectively, in a pond with considerable flora. Several lines of evidence suggest that, even when a substantial O_2 partial pressure gradient exists, O_2 availability from the embryonic environment is equally nonlimiting to aerobic metabolism in embryos in the center compared with those at the surface of the egg mass. First, lactate levels were consistently low in embryonic tissue from all depths in the egg mass. In accord with this finding was the tendency for pH of egg jelly in the center of the mass to be only very slightly depressed from that measured at the egg mass surface. Elevated lactate and depressed pH levels probably would develop if conditions within the egg mass favored anaerobic metabolism. In addition, embryonic O_2 consumption was unaffected by ambient P_{O_2} to levels well below the lowest P_{O_2} recorded in the center of the intact egg mass. Thus, even if the center of the egg mass became substantially hypoxic, there would be no direct effect upon O_2 uptake by embryonic tissue, at least in the short term. Finally, embryonic O_2 consumption was not significantly affected

removal of the eggs from the egg mass, which essentially placed all embryos in conditions for gas exchange equivalent to those experienced by embryos at the edges of the egg mass. This finding is consistent with the hypothesis that aerobic metabolism is not limited by the normal degree of O_2 depletion deep within the mass. Collectively, these data suggest that embryos in the center of egg masses laid by *Rana palustris* have the same ratio of aerobic to anaerobic metabolism as those directly exposed to the environment at the egg mass surface.

The present findings are in distinct contrast to earlier studies on spherical egg masses of other ranid frogs (Savage 1935; Barth 1946; Gregg 1962), which presented direct or indirect evidence for critically hypoxic or nearly anoxic conditions at egg mass centers. Typically, these earlier experiments were performed on eggs that had been removed from the original egg mass, stripped of egg jelly, and then tightly layered together on the bottom of glass dishes. One purpose of jelly in invertebrate egg masses is simply to separate the embryos to facilitate gas exchange by diffusion (Strathmann and Chaffee 1985). Whether this is true of amphibian egg masses is not clear, but in any event there are probably few parallels between respiratory conditions surrounding embryos in the natural egg mass and those of embryos that have been handled in any fashion.

Though I anticipated a reasonable degree of oxygenation within the egg masses, the finding of conditions nearing air saturation in the center of some large masses was unexpected. Two characteristics of the nest contribute to this. First, though no convection of the jelly occurs, the jelly is a relatively permeable barrier to O_2 diffusion—relative to muscle or connective tissue, for example. Moreover, Krogh's constant for O_2 diffusion in jelly is 75% that of distilled water at identical temperature. Consequently, conditions of oxygenation within the center of the egg mass would not be radically different from those in a group of equally spaced eggs surrounded by stagnant water.

The second feature of the egg mass that makes an important contribution to gas exchange between edge and center, as well as to gas exchange throughout the egg mass, involves fluid convection within the eggs. The ciliated epithelium of the embryo generates a substantial current of fluid within the egg. This current actively ventilates the embryo's body surface, across which all gas exchange occurs until the development of gills immediately before hatching (see Burggren 1984). Not only is stagnation of egg fluid at the embryonic surface prevented, but the path of the current brings fluid within close proximity to the surface of the egg. This flow of extra-embryonic fluid almost certainly aids O_2 and CO_2 transport between the embryonic tissues and the environment and is somewhat analogous in function to the chorioallantoic circulation in eggs of higher vertebrates. Interestingly, cilium-induced convection of egg fluid also occurs in many invertebrate eggs and has been implicated in gas exchange in the extensive egg masses laid by opisthobranchs and some prosobranchs and polychaetes (Strathmann and Chaffee 1985).

The velocity of the cilium-induced fluid current within the eggs of *Rana palustris* is maintained in the face of reduced O_2 levels and increases substantially with temperature. Since aerobic metabolism also continues during hypoxia and increases with temperature, these ciliary responses are consistent with a gas-exchange function. As Strathmann and Chaffee (1985) have indicated for opisthobranch veligers, it has yet to be determined whether these cilium-induced changes in convection of egg fluid compensate for the metabolic cost of ciliary motion under these circumstances.

This organized pattern of fluid convection within the frog egg has important implications for gas exchange throughout the egg mass. Rather than modeling the egg mass as an immobile, embryo-studded sphere with all gas exchange with central regions occurring by passive diffusion, the egg mass of *Rana palustris* should be viewed as a sphere embedded with several hundred equally spaced pockets of intensive convection. Convection not only will assist gas exchange within each egg capsule but may also contribute actively to the exchange of gases between the deeper regions of the egg mass and its surface. This

latter suggestion rests on several simplifying assumptions. The first assumption is that bulk transport of gases within the egg fluid occurs by convection rather than diffusion. Second, it is assumed that substantial amounts of oxygen diffusing into the egg capsule at the side of the capsule facing the edge of the egg mass are rapidly transmitted by convection to the point on the egg capsule membrane facing the interior of the mass (fig. 2), there to diffuse out according to the P_{O_2} gradient across the capsule membrane, *before* being consumed by the embryo. That is, convection in the egg actually produces a net *transport* of O_2 into the egg, across its diameter, and out the other edge. Finally, it is assumed that the egg "capsule" or membrane is not a major barrier to gas diffusion. The gas-diffusion properties of the amphibian egg capsule have not been measured, but the capsule can be highly permeable to water and solutes (Holtfreter 1943).

Based on these assumptions, the gas diffusion distance between surface and center of a hypothetical spherical egg mass will be equal to the egg mass radius minus the distance along this radius occupied by eggs rather than jelly. This distance can be inferred from the relative volume of eggs to total egg mass. Given a hypothetical egg mass with dimensions equivalent to the mean values reported above (radius = 44.5 mm, n embryos = 959, egg diameter = 5 mm), it can be calculated that 17% of the volume of the mass is actually egg. Thus, an average of 17% of the line scribing the radius will be occupied by egg rather than jelly.

This simple analysis indicates that gas transfer occurs by convection rather than by diffusion along approximately 17% of the total distance from the surface of the egg mass to its center. This "ventilation" of the inner regions of the egg mass provided by convection within individual eggs potentially contributes to the aerobic conditions at the egg mass center. Experimental support of this hypothesis would require eliminating ciliary motion of the embryo's surface without simultaneously affecting its metabolic rate and observing whether respiratory conditions within the egg mass deteriorate when all gas exchange is relegated to diffusive processes. Unfortunately, currently known ciliary motion uncouplers also affect cellular metabolism.

Finally, Petranka et al. (1983) have suggested that the eggs of the salamander *Ambystoma opacum* and the frog *Rana palustris* will not hatch if the eggs are maintained at an ambient P_{O_2} above 80–90 mmHg. These authors suggest that embryonic hypoxia, modified by adjustments in pH, is the physiological trigger to hatching. The importance to the hatching of amphibian eggs of an ambient pH below neutral has also been emphasized by Pough (1976). Yet, the present experiments on one of the same ranid species studied by Petranka et al. (1983) indicates that normal development and hatching occur when at least the normal, undisturbed environment immediately around each egg nears air saturation, rather than only under hypoxic conditions as Petranka et al. (1983) reported.

Clearly, there is still much to be learned about interspecific and environmental influences on the highly complex process of embryonic development in amphibian egg masses.

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