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Morphometry and estimated bulk oxygen diffusion in larvae of *Xenopus laevis* under chronic carbon monoxide exposure

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**Abstract** To understand the mechanisms that allow tadpoles of the African clawed frog *Xenopus laevis* to develop under conditions of impaired convective transport (hemoglobin poisoning with carbon monoxide), whole animal surface area and volume were measured and bulk oxygen diffusion was modeled at four developmental stages (from initiation of heartbeat to premetamorphic climax). Surface area \( [8.5 \, \text{mm}^2 \text{ at stages Nieuwkoop-Faber (NF) 33–34 to 70.2 \, \text{mm}^2 \text{ at stages NF 50–51}] \) and volume \( [1.8 \, \text{mm}^3 \text{ at stages NF 33–34 to 35.7 \, \text{mm}^3 \text{ at stages NF 50–51}] \) measured from volumetric analysis from dual plane images of each animal were not significantly different between treatments. Bulk oxygen radial diffusion was estimated by modeling the larvae as a set of adjacent cylinders with different radii. The model was used to predict the oxygen tension at the water-skin interface at which the oxygen tension in the center of the animal is nil \( (0.7 \, \text{kPa at stage NF 33–34 and 14.0 \, \text{kPa at stage NF 50–51])}, \) suggesting that bulk oxygen diffusion is sufficient to meet the metabolic demand up to stages NF 46–47 irrespective of the oxygen tension at the water-skin interface. At NF 50–51 an anoxic core in the animal would appear if bulk oxygen diffusion were the only means of oxygen transport at oxygen tensions below 15 kPa. However, the relative volume of the anoxic core would only exceed 10% of the total volume of the animal only at oxygen tensions below 5 kPa. Therefore, the ten-fold increase in mass between NF 50–51 and metamorphosis would prove insufficient for embryonic oxygen requirements via simple diffusion, and therefore would require additional transport mechanisms.

**Key words** Surface area · Diffusion model · Gas exchange · Ontogeny · Amphibian

**Abbreviations** \( K \) Krogh diffusion coefficient · \( m \) volume-specific oxygen consumption · \( M_O_2 \) oxygen consumption · \( NF \) Nieuwkoop-Faber · \( P_o \) partial pressure of oxygen at the surface of the animal · \( P_O_2_{min} \) oxygen tension at the water-skin interface that resulted in a nil oxygen flow at the core · \( R \) radius of each slice · \( R_{max} \) largest distance from the surface of the animal to the core · \( r_p \) radius of the cylinder at which diffusive oxygen flux is zero · \( SA \) \( V^{1/3} \) ratio of surface area to volume

**Introduction**

The need for oxygen has been long assumed to be the driving force behind the development of the cardiovascular system. In this view, convective transport of oxygen would begin just as diffusion becomes inadequate because of lengthening diffusion distances and increasing metabolic rates (Adolph 1979; Boell et al. 1963; Burggren and Pinder 1991; Burggren and Territo 1996). However, recent studies in fish and amphibian larvae using CO-poisoning or chemical destruction of blood cells have challenged this concept by demonstrating that oxygen requirements are not strictly coupled to the convective transport of respiratory pigments (Pelster and Burggren 1996; Territo and Altimiras 1998; Territo and Burggren 1998). Also, mutant axolotls in which the heart never starts to beat are able to hatch and swim...
(Justus 1978) and have a similar oxygen consumption to their normal counterparts (Mellish et al. 1994).

The uncoupling between oxygen demand and oxygen transport could be due to a large functional reserve for gas exchange in larvae because bulk oxygen diffusion suffices to meet metabolic demand even when convective transport is blunted. Alternatively, impaired larvae could follow a different developmental trajectory in order to increase oxygen uptake. This could be realized by increasing direct diffusion via morphologic changes and/or by increasing convective transport. However, in earlier studies on CO-poisoned *Xenopus laevis* larvae, only small changes in cardiac output compared to controls were observed, suggesting that convective transport of oxygen in plasma is not responsible for the maintained oxygen uptake (Territo and Altimiras 1998; Territo and Burggren 1998).

The current study was designed to estimate the role of morphometric alterations in total body shape in *X. laevis* under chronic exposure to carbon monoxide and to develop a model to evaluate the role of bulk oxygen diffusion to the tissues under conditions of impaired oxygen transport.

**Materials and methods**

Experimental animals and developmental conditions

Newly laid fertile eggs were obtained by breeding four adult female *X. laevis* (Thompson and Franks 1978). Eggs were placed into two 25-l holding tanks, where they were kept in dechlorinated water at 24 ± 0.2 °C, 14:10 light/dark photoperiod and fed Nasco frog brittle ad libitum.

Two different experimental groups were established based in two different aeration regimes: normoxia (21 kPa O$_2$/balance N$_2$) and CO exposure (2 kPa CO/21 kPa O$_2$/balance N$_2$). The aeration regime with CO has been shown to reduce the oxygen content of the blood below 1%, in comparison with oxygen contents during normoxia ranging from 8–15% depending on the hemoglobin content of the blood (Territo and Burggren 1998).

The study was carried out at four developmental stages: Nieuwkoop-Faber (NF) 33–34, NF 44–45, NF 46–47, and NF 50–51. NF 33–34 embryos (20.5 h posthatching, average length of 4.7–5.3 mm) were chosen because the heart starts beating at this stage. In NF 44–45 larvae (3–4 days-old, average length 7.8–10 mm) circulation in the external gills peaks at this stage, the heart is septated and the cardiac valves are fully developed. NF 46–47 larvae (5–days-old, average length 9–15 mm) mark the end of the degeneration of the external gills. At NF Stage 50–51 (15- to 17-days-old larvae, length range 20–36 mm) the lungs appear and hindlimb buds are clearly visible.

**Videotaping and calibration for surface area and volume**

Six animals from each of the two experimental treatments and four developmental stages were sampled from the experimental tanks, anesthetized with buffered MS-222 (0.02%, w/v) and transferred to an agar plate. The earlier developmental stages (NF 33–34 and NF 44–45) were videotaped under a Leica M3Z compound microscope fitted with a color video camera (Javelin Electronics). Later stages (NF 46–47 and NF 50–51) were videotaped using a compact VHS video-camera (model GR-AX9000U, JVC) attached to a tripod. Each animal was taped in the dorsal and lateral views insuring that both planes were perpendicular to one another and to the mid-line of the animal. In order to calibrate the videotaped images, a piece of polyethylene tubing of well-defined dimensions (see Image analysis section) was recorded along with each treatment group.

**Image analysis**

The images were played back on a Panasonic SVHS editing VCR (Model AG-7350) connected to a computer image analysis system based on OPTIMAS software. Calibration was performed using the recorded PE-10 tubing (Becton-Dickinson) of known dimensions (D.O. = 610 μm). The perimeter of the animal was delineated on the computer screen using the computer mouse and stored in the form of Cartesian coordinates in an Excel spreadsheet with a 10-μm resolution.

**Determination of total surface area and total volume of the larvae**

Total surface area and total volume were estimated by virtually splitting the animal in cross-sectional slices of a fixed width. Volume and surface area for each slice were then calculated and summed to give an estimate of total volume and total surface area of the larvae. Each slice was modeled as an elliptical cylinder using the thickness of the slice as the height of the cylinder. Considering each slice as an elliptical cylinder largely underestimated surface area, so the surface area between the anterior and posterior edges of each slice needed to be determined. For details on the equations used in surface area and volume calculations see the Appendix section.

All computations were performed by a custom-designed program using a graphical programming environment (LabView, National Instruments). The Cartesian coordinates of each larva were used to obtain three profiles: dorsal, ventrolateral and dorsolateral. The dorsal plane is symmetric and only the right half side was used. The lateral plane is conspicuously asymmetric and both halves were obtained and used in the analysis. Before the application of the algorithm, three correction routines were employed to ensure a correct alignment of the three profiles of the animal:

1. Normalization of the x-axis to adjust the three profiles to the same length.
2. Correction for the pitch of the animal by linear regression.
3. Interpolation in each profile to obtain an evenly spaced sequence of Cartesian coordinates.

**Verification of the algorithm**

Because the accuracy of the method is largely dependent on the choice of the slice thickness, a preliminary test was carried out to define an appropriate thickness (see Results). Computer-generated geometrical models for which volume and surface area are known through classical geometry were employed. These included spheres, spheroids (prolate and oblate), ellipsoids and cones of different dimensions, matching the size of the experimental animals.

Once the slice thickness was selected, the new method was evaluated. The calibration aimed to determine the accuracy of the entire procedure, which includes the image analysis system and the manual delineation of the animal on the computer screen. The volume and surface area of three metal spheres (2.32, 3.15 and 6.12 mm nominal diameters; Small Parts, Miami, Fla.) were determined. Each was videotaped, as previously described, stored and analyzed.

**Description of the diffusion model**

The diffusion of oxygen through living tissue is described by the general form of Fick’s first law of diffusion (Withers 1992) and