

Respiratory Gas Exchanger Partitioning in *Ambystoma mexicanum* during an Aquatic Hypoxic Challenge

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The Mexican salamander, or axolotl (*Ambystoma mexicanum*), is a neotenic urodele native to the lake district around Mexico City (approximately 6000 ft.). It is an ectothermic bimodal breather from altitude and this uniquely suits it to study in adaptation to environmental hypoxia (low-oxygen stress).

Because the axolotl is a bimodal breather and is constantly faced with acute and chronic environmental hypoxia in its native surroundings, the effective partitioning of its gas exchange (choosing to ventilate gills, lungs or both) during such stress may allow this animal to thrive in these hypoxic extremes. Presumably, the oxygen level during daylight in the lakes that the animals inhabit is only moderately hypoxic due to altitude. At night, however, the photosynthetic O₂ producers shut down and oxygen levels drop precipitously, thus placing the animals in a severely hypoxic environment.

The questions we set out to address were:

1) What contributions to total oxygen consumption do the lungs and gills make during an aquatic hypoxic challenge? and 2) What is the critical aquatic oxygen tension (PO₂) at which the aerial exchanger is used in preference to the aquatic exchanger?

Materials and Methods. Three groups from a normal population of adult neotenic male axolotl salamanders were used. They were obtained from the Indiana University Axolotl Colony and kept in a temperature and photoperiod controlled environmental chamber.

Animals in the first group (n=11) were placed in a temperature controlled, closed-flow metabolic chamber, and their aquatic oxygen consumption was measured using an oxygen electrode. Aerial consumption was measured separately in an open flow, temperature controlled metabolic chamber using

an oxygen analyzer. PO₂ in the open flow chamber was maintained at the desired tension through the use of a gas mixing pump.

Animals in the second group (n=7) were placed in a temperature controlled, closed-flow metabolic chamber designed specifically for simultaneous oxygen consumption analysis. This system was similar to the system used by Whitford et al. in their work with *A. tigrinum* (Whitford et al, W., 1968). Aquatic consumption was measured by the use of an oxygen electrode. A water recirculating pump was employed in series with the system to insure a uniform dissolved oxygen presentation to electrode. Aerial oxygen consumption was measured from a free, room air/water surface using an oxygen analyzer. In order to limit oxygen diffusion across this interface, a 0.5 cm layer of mineral oil separated air from water.

Animals in the third group (n=5) were placed in a modified version of the simultaneous closed-flow metabolic chamber. This chamber contained a port for the passage of a branchial arterial cannula. The cannula was inserted into the branchial artery on the third right gill raker after the animal was anesthetized in a .03% benzocaine solution per a previously reported method (Zwemer, C., 1991). The animal was restrained in fiberglass screen "trousers" and upon revival was placed into the chamber for data collection following the previously described protocol.

Blood samples were withdrawn during hypoxia and not allowed to equilibrate with ambient air via the cannula during experimentation. Whole blood was also analyzed for hematocrit (HCT), hemoglobin concentration [Hb], and oxygen content. Hematocrit was determined using a microcentrifuge, [Hb] with a standard Sigma kit, and oxygen content with a Roughton-Scholander microgasometric analyzer.

Results. Animals denied an air surface demonstrated a significantly reduced oxygen consumption with a decreasing aquatic PO₂ while animals allowed access to the air surface demonstrated a significant increase in consumption (Figure 1).

When measured simultaneously, animals clearly demonstrated partitioning of their exchangers. Below a PO₂ of 50 mm Hg (Figures 2 & 3) the animals relied almost entirely on lung ventilation for O₂ consumption, while the gills contributed minimally. Total oxygen consumption decreased as hypoxic exposure

Figure 1. Oxygen consumption Aquatic and Aerial

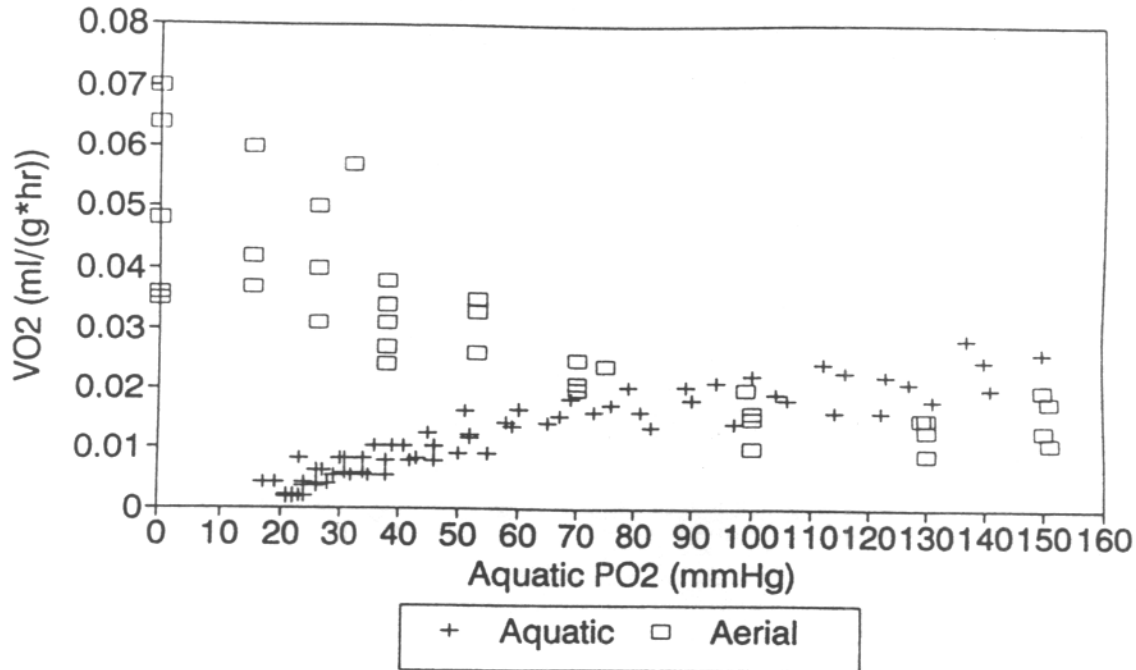


Figure 1. Animals denied an air space decreased aquatic O₂ consumption (VO₂) with decreasing aquatic PO₂. When allowed access to the air surface, aerial consumption increased with aquatic PO₂ decrease.

Figure 2. Simultaneous Oxygen Consumption (VO₂)

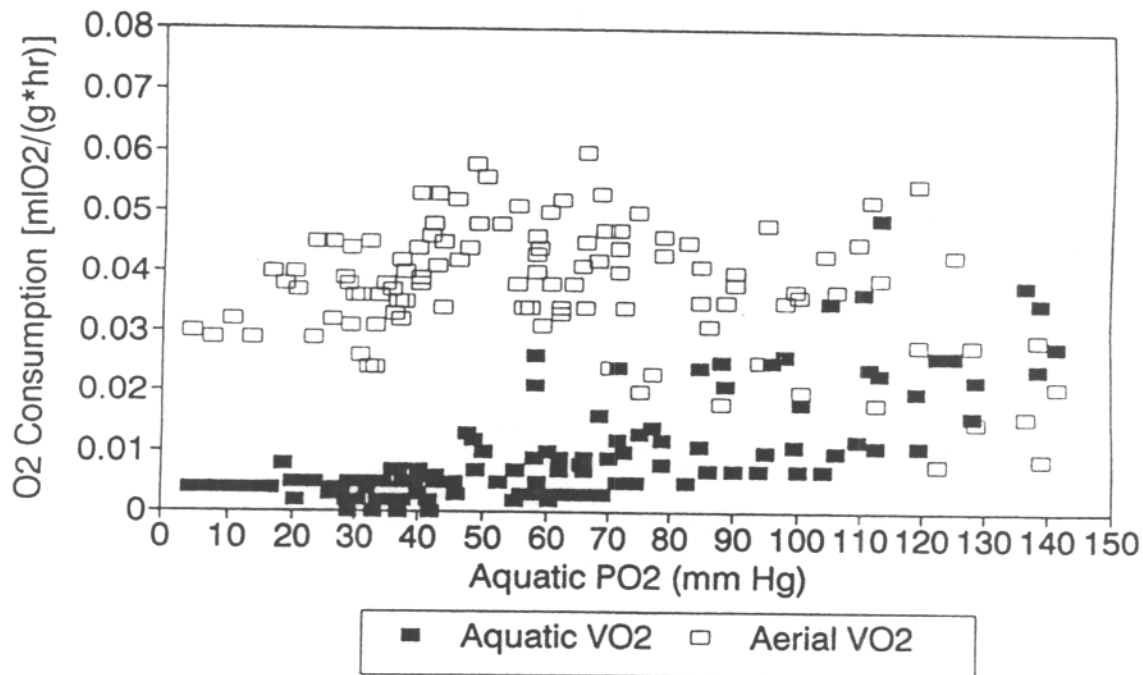


Figure 2. With decreasing aquatic PO₂, aquatic O₂ consumption decreases while aerial O₂ consumption increases.

became more severe, and mixed venous blood O_2 content followed this trend (Figures 4 & 5).

Discussion. Animals denied access to a free air space during a progressive hypoxic challenge clearly decreased oxygen consumption as compared to animals allowed access to the airspace (Figure 1). The decreased consumption of oxygen with increasing depth of hypoxia during exclusive aquatic ventilation has been demonstrated before in *Necturus maculosus* (Shield, J. et al., 1973). This decrease in consumption suggests that when challenged by aquatic hypoxia, oxygen consumption is a function of the availability of access to air and that metabolism may be depressed when axolotls are forced to breathe water exclusively.

The simultaneous recording of aerial and aquatic oxygen consumption in the axolotl indicated that with a decreasing aquatic PO_2 and constant aerial PO_2 (150 mm Hg), the animal began to rely increasingly on its aerial gas exchanger (Figs. 2 & 3). Between PO_2 's of 150 to 100 mm Hg, no important difference in exchanger contribution to O_2 consumption existed. However, between PO_2 's of 100 and 56 mm Hg the contribution of aerial gas exchange increased and, at PO_2 's below 55 mm Hg, the aerial exchanger accounted for 85 to 90% of the oxygen uptake. Clearly, the axolotl had effectively partitioned its exchangers. These results are similar to those reported by Heath in which the contribution of gas exchangers to O_2 consumption in *A. tigrinum*

changed with increasing depth of hypoxia (Heath, A., 1976). Presumably this switching from water breathing to air breathing allowed the axolotl to defend its tissue PO_2 in the face of extreme environmental hypoxia.

Total oxygen consumption of the axolotl during a progressive hypoxic challenge appeared to decrease with decreasing PO_2 , thus suggesting that even with access to a free air space metabolism may be depressed with increasing aquatic hypoxia (Fig. 4).

Preliminary results from cannulation studies indicated that with decreasing aquatic PO_2 , total oxygen consumption is related to blood oxygen transport (Fig. 5). This suggests that by increasing blood transport parameters such as hematocrit and hemoglobin concentration (Table 1), the animal would be able to tolerate a greater depth of hypoxia. An increased blood oxygen capacitance would allow more oxygen to be extracted and delivered to the tissues thus defending tissue PO_2 . Further studies on hypoxically adapted animals are required however to verify this inference.

In conclusion, it is clear that the Axolotl effectively partitions its respiratory gas exchangers in response to a hypoxic challenge. As aquatic PO_2 falls, exchange at the lungs increases in importance as reliance on the gills diminishes. Total oxygen consumption diminishes slightly. At PO_2 's below 50 mmHg, the lungs are responsible for nearly all O_2 consumption. All of these responses may facilitate the protection tissue PO_2 .

Table 1. Results

	Mean	Standard Deviation
Mean Total Oxygen Consumption	0.047 ml O_2 /(g*h)	0.011
Mean Mass	153 g	28
Mean Hematocrit	33%	5.7
Mean Hemoglobin Concentration	8.9 g/dl	3.0
Mean Corpuscular Hemoglobin Concentration	26.8 g/dl	6.0

Figure 3. Partitioning of Gas Exchange
Percent Contribution to Total VO₂

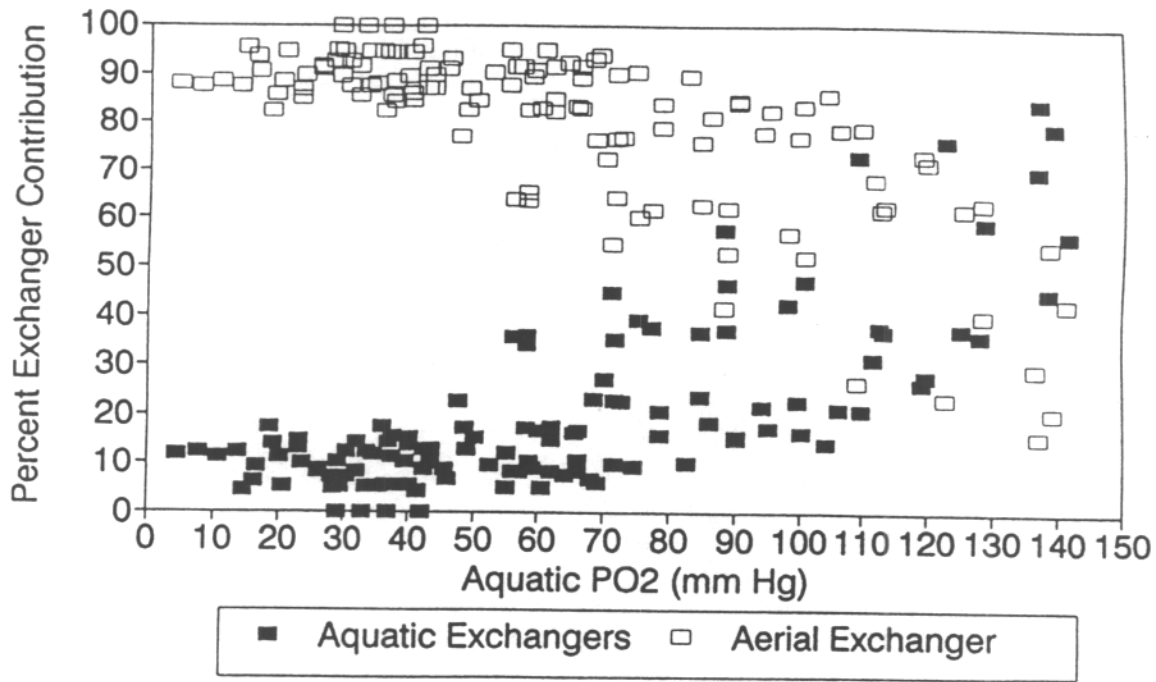


Figure 3. As aquatic PO₂ drops, the axolotl relies increasingly on its aerial exchanger (lungs) for gas exchange, while it decreases its reliance on the aquatic exchanger (gills).

Figure 4. Total Oxygen Consumption

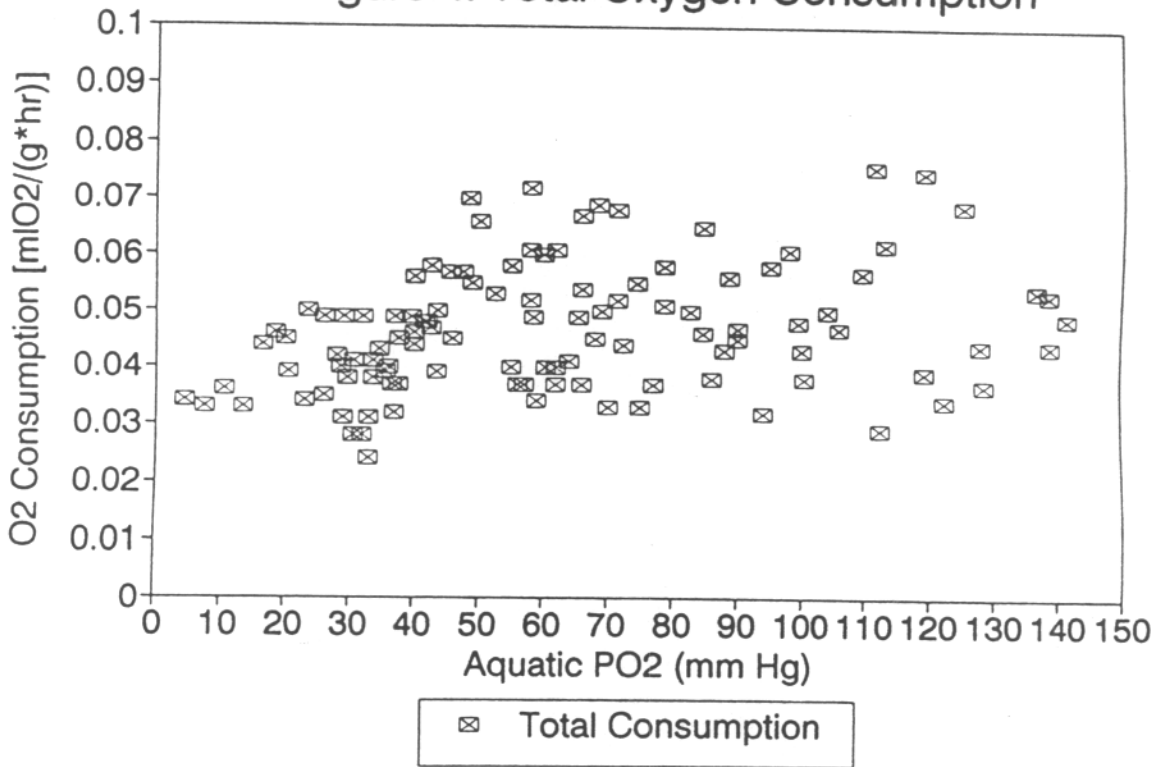


Figure 4. With decreasing aquatic PO₂, total consumption remains relatively unchanged. It does however demonstrate a decreasing trend as aquatic PO₂ drops below 50 mm Hg.

Figure 5. Blood O₂ Content and Total Oxygen Consumption (VO₂) vs. PO₂

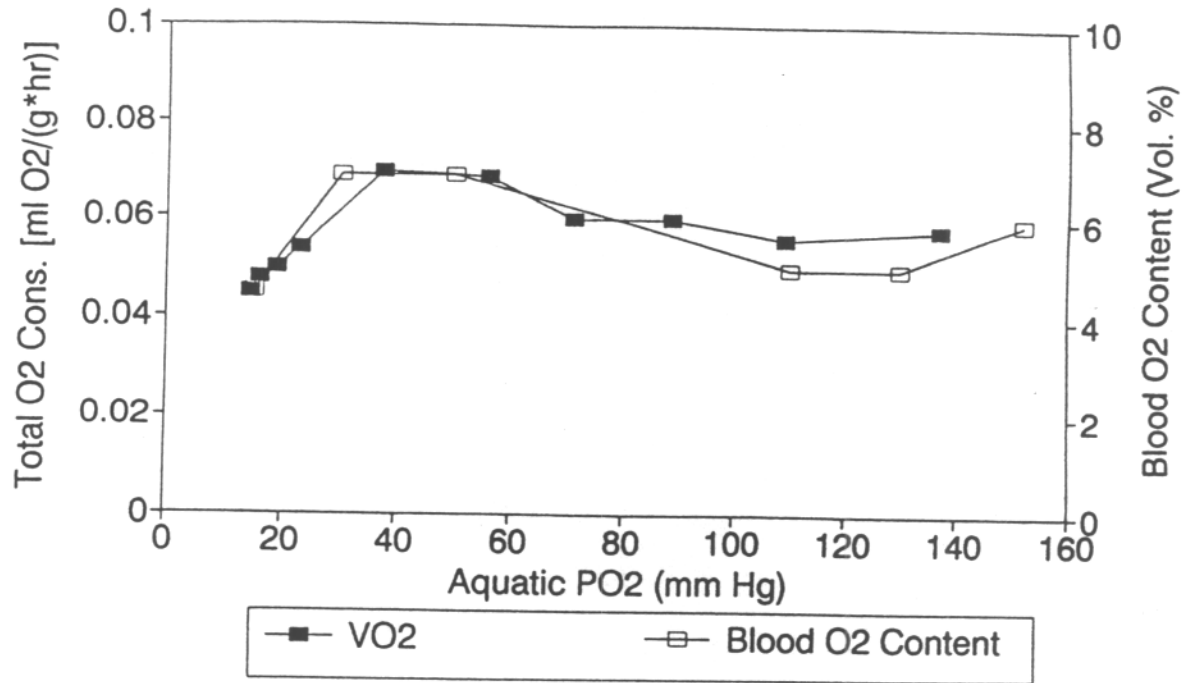


Figure 5. Preliminary data from one animal demonstrating blood O₂ content with decreasing aquatic PO₂. Total O₂ consumption vs. PO₂ is also presented, demonstrating a possible delivery limited system.

Literature Cited

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