

AQUATIC OXYGEN CONFORMITY IN THE MEXICAN AXOLOTL, *AMBYSTOMA MEXICANUM*

H.I. GRIFFITHS¹ AND D.H. THOMAS²

¹ *Environment Lab., School of History and Archaeology, University of Wales College of Cardiff, P.O. Box 909, Cardiff, CF1 3XU, U.K.*

² *School of Pure and Applied Biology, University of Wales College of Cardiff, P.O. Box 915, Cardiff, CF1 3TL, U.K.*

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ABSTRACT

The Mexican axolotl, *Ambystoma mexicanum* is an aquatic-phase oxygen conformer with a close correlation between rates of aquatic oxygen consumption and aquatic oxygen tension. Rates of oxygen consumption at normoxic oxygen tensions were 18.2 $\mu\text{l O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ at 20°C and 13.8 $\mu\text{l O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ at 30°C. Air-breathing (i.e. rising to the water surface to gulp air) increases in hypoxia, associated with a decrease in gill ventilatory movements. Therefore, oxygen compensation during aquatic hypoxia appears to occur in the aerial phase, whilst aquatic gas-exchange surfaces show little ability to compensate for changes in ambient oxygen tension.

INTRODUCTION

Three major strategies for respiration have been identified for primarily aquatic urodeles by Guimond and Hutchison (1973). These are the development of elaborate gills with poorly developed and vascularised lungs as in the Proteidae, air-breathing with elaborate lungs but with the gills vestigial or absent as in the Sirenidae and the Amphiumatidae, or a lack of either elaborate gills or lungs but with gas-exchange taking place primarily across a highly vascularised and expanded skin surface as in the Cryptobranchidae. The facultatively paedomorphic members of the Ambystomatidae would seem to represent a fourth option however, having primitive but functional lungs (Brunst, 1955) and well-developed external gills (McIndoe and Smith, 1984). Both aerial and aquatic respiration have been shown to contribute significantly to the animals needs (e.g. Whitford and Sherman, 1968; Lenfant, Johansen and Hanson, 1970; Heath, 1976).

The relative importance of the different gas-exchange sites (gills, lungs, skin) varies between amphibians. There is much evidence that cutaneous respiration can be the most significant aquatic gas-exchange surface in many adult urodeles; in *A. mexicanum* the skin of the back and flanks is thin and well-vascularised (Czopek, 1957), whilst the tail is expanded dorsally and ventrally into fins, these adaptations serving to reduce diffusive distances and to increase the surface area available for aquatic gas-exchange (see Feder and Burggren, 1985). Bucco-pharyngeal mucous membranes are not usually considered competent for gas-exchange in urodeles because of the poor development of the intraepithelial capillaries (Elkan, 1955, 1958). In larval and paedomorphic forms the gills appear to function primarily in acid-base regulation and in carbon dioxide exchange (Baldwin and Bently, 1982; Lewinson, Rosenburg, Goldenburg and Warburg, 1987; Boell, Greenfield and Hille, 1963).

The object of this study was to observe the effects of a variety of aquatic oxygen tensions on the rate of cutaneous uptake of oxygen by *A. mexicanum*, to determine the role of aquatic gas-exchange under different ecophysiological conditions.

MATERIALS AND METHODS

Ten paedomorphic albino *A. mexicanum* of similar age (weight range 85g-106g) were obtained from a local breeder. Animals were kept in two large aquaria and maintained at ambient temperature on a 12 hour light: 12 hour dark photoperiod. Animals were starved for three days prior to use and rested for several days between experimental runs. Identification of individual animals was facilitated by marking with a fish-tattoo panjet (F.H. Wright, Dental Manufacturing Co. Ltd., Dundee) loaded with alcian blue dye (Wisniewski, Paull, Merry and Slater, 1980). Animals were acclimated to experimental temperatures overnight before use. Stocks of hypoxic and hyperoxic maintenance media were made up from oxygen-saturated and oxygen-free stock prepared by bubbling gaseous oxygen or nitrogen through bulk water samples under liquid paraffin (B.D.H. Ltd.).

Respirometry was carried out in a closed system within a heated water bath at 20°C or 30°C with media (1 litre) of various initial oxygen tensions made by mixing hypoxic and hyperoxic stocks in the appropriate proportions. Animals were placed in experimental chambers and the air/water interface sealed with a thin layer of liquid paraffin so as to isolate the water phase from atmospheric oxygen without restricting the animals access to air.

After allowing animals to settle, water samples were taken at thirty minute intervals over a period of two hours. Three 20ml water samples were removed in 25ml disposable syringes adapted to allow the addition of titration reagents, whilst excluding atmospheric oxygen. Sample volumes were replaced with oxygen-purged water introduced through the paraffin layer. The oxygen content of the samples was measured by a Winkler titration method modified from that of Hoar and Hickman (1967). Aquatic oxygen concentrations were converted to $P_{\text{O}_2(\text{w})}$ using the α -values for freshwater from Randall (1970). The coefficient of variation of dissolved oxygen concentrations obtained by this method during control runs was 5.6%. Experimental runs in which animals showed clear escape reactions were excluded from data analysis. Virtually all respirometry was carried out between 09.00 and 13.00 hours to reduce diel effects in oxygen consumption.

previous observations having shown that *A. mexicanum* displays diel variation in ventilatory cycles (Griffiths and Thomas, unpublished data).

Oxygen consumption rates were calculated from slopes of oxygen concentration against time (determined by least squares regression) and expressed as $\mu\text{l O}_2(\text{S.T.P.D.})\cdot\text{gram wet body mass}^{-1}\cdot\text{hour}^{-1}$.

The effect of aquatic oxygen concentration on the frequency of lung and gill ventilations was also examined by direct observation. Animals were held at 20°C in a constant temperature room and the total number of lung ventilations per hour recorded. Gill ventilatory movements were recorded as the mean number of gill beats per minute in each of four random one minute periods within each hour.

The relationships between aquatic oxygen tension ($P_{\text{O}_2(\text{w})}$) and the rate of oxygen consumption ($\dot{V}_{\text{O}_2(\text{w})}$), gill ventilation frequency (f_{gill}) and lung ventilation frequency (f_{lung}) were examined by linear least-squares regression analysis.

RESULTS

There was a strong positive correlation between the rate of oxygen consumption and the oxygen tension of the water at both experimental temperatures. The relationship between $\dot{V}_{\text{O}_2(\text{w})}$ and initial oxygen tension at 20°C and 30°C is shown in Fig. 1 and regressions at each temperature are shown below. The strongly positive slopes show that *A. mexicanum* is an oxygen conformer for its aquatic-phase respiration, at least in this temperature range:

At 20°C:

$$\dot{V}_{\text{O}_2(\text{w})} = (-2.29 \pm 1.99) + (0.13 \pm 0.03) \cdot P_{\text{O}_2(\text{w})(\text{initial})}$$

Units: $\mu\text{l O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ & mmHg; $r = 0.92$, $df = 14$, $P < 0.001$.

At 30°C:

$$\dot{V}_{\text{O}_2(\text{w})} = (-1.08 \pm 1.02) + (0.09 \pm 0.03) \cdot P_{\text{O}_2(\text{w})(\text{initial})}$$

Units: $\mu\text{l O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ & mmHg; $r = 0.99$, $df = 8$, $P < 0.01$.

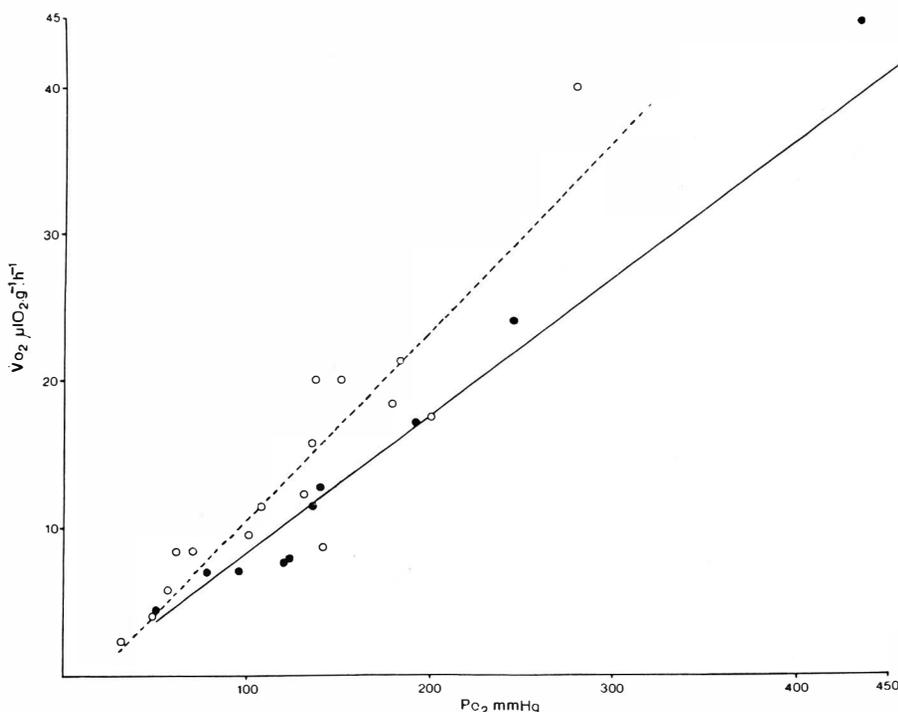


Fig. 1 Rates of oxygen consumption of axolotls in water of differing initial aquatic oxygen tensions at 20°C (broken line and open circles) and 30°C (solid line and closed circles). Regressions at 20°C and 30°C are given in the text.

$$f_{\text{gill}} = (1.17 \pm 1.70) + (0.02 \pm 0.01) \cdot P_{\text{O}_2(\text{w})(\text{initial})}$$

Units: $f_{\text{gill}} \cdot \text{min}^{-1}$ & mmHg; $r = 0.42$, $df = 13$, $P > 0.05$.

$$f_{\text{lung}} = (6.12 \pm 2.43) + (0.03 \pm 0.02) \cdot P_{\text{O}_2(\text{w})(\text{initial})}$$

Units: $f_{\text{lung}} \cdot \text{hr}^{-1}$ & mmHg; $r = 0.48$, $df = 13$, $P > 0.05$.

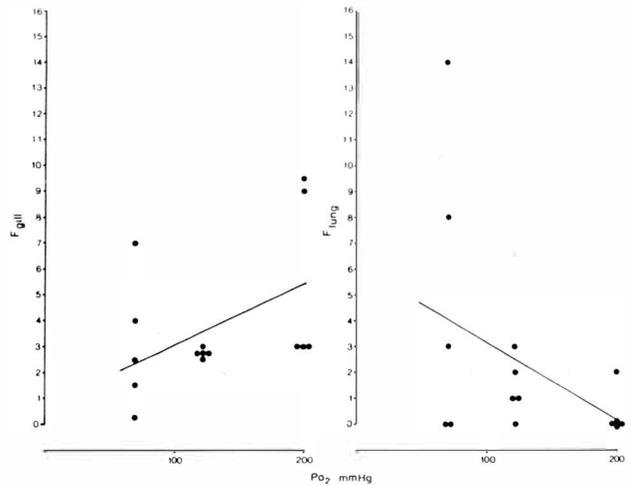


Fig. 2 Frequency of gill ventilation ($f_{\text{gill}} \cdot \text{minute}^{-1}$) and lung ventilation ($f_{\text{lung}} \cdot \text{hour}^{-1}$) of axolotls under different aquatic oxygen tensions at 20°C. Regressions are given in the text.

Axolotls appear to compensate for aquatic hypoxia by increasing aerial respiration. Fig. 2 shows apparent trends for f_{gill} to decrease with decreasing aquatic oxygen tension and for f_{lung} to increase, although neither of these regressions is statistically significant:

DISCUSSION

The results presented here show that *A. mexicanum* is an aquatic-phase oxygen conformer: in other words, in water oxygen uptake is determined by external $P_{\text{O}_2(\text{w})}$, and the animal has little ability therefore to regulate its O_2 gains. Previous similar studies of paedomorphic aquatic salamanders are sparse, although Heath (1976) has reported aquatic-phase oxygen conformity in paedomorphic *Ambystoma tigrinum*. On the other hand, Branch and Taylor (1977) found *A. maculatum* to be oxygen conformers only before metamorphosis, whilst post-metamorphic animals were oxygen regulators (i.e. the animal itself can take measures to compensate for variable external $P_{\text{O}_2(\text{w})}$ by increasing the effectiveness of O_2 uptake processes when $P_{\text{O}_2(\text{w})}$ is low). Ultsch (1973) reported *Siren lacertina* of a variety of size classes to be oxygen conformers at oxygen tensions below 80mmHg, above which they became oxygen regulators. From our data there is no evidence of aquatic oxygen regulation in *A. mexicanum* over a broad range of aquatic oxygen tensions, therefore any compensation for environmental hypoxia must occur in the aerial phase.

Rose, Armentrout and Roper (1971) have shown that paedomorphs of *A. tigrinum* are remarkably resistant to enforced aquatic anoxia and are apparently capable of anaerobiosis, as indicated by reduced cardiac glycogen stores and increased blood lactate and glucose levels. *A. mexicanum* may also be capable of withstanding environmental anoxia and studies of muscle histology that report poor capillarisation of the white muscle fibres lend support to this view (Totland, 1984). However, behavioural avoidance, if possible, appeared to over-ride physiological compensation; in severe hypoxia a number of our animals showed profound escape responses, to the extent of climbing out of the experimental chambers into the surrounding water-bath.

Our value for the rate of (normoxic) aquatic oxygen consumption at 20°C ($18.2\mu\text{l O}_2\cdot\text{g}^{-1}\cdot\text{h}^{-1}$, $P_{\text{O}_2(\text{w})} = 155\text{mmHg}$) is consistent with values of $22\mu\text{l O}_2\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ for *A. mexicanum* at 20°C reported by Gahlenbeck and Bartels (1970). The apparent interspecific difference in $\dot{V}_{\text{O}_2(\text{w})}$ is probably a simple effect of body mass: assuming a scaling exponent of -0.34 for mass-specific metabolism in aquatic urodeles (cf. Ultsch, 1974), $\dot{V}_{\text{O}_2(\text{w})}$ for animals of body mass 44g (Gahlenbeck and Bartels, 1970) and 100g (this study) would be expected in proportion of 24:18, which is close to the observed proportions in the two studies. More precise comparisons are not possible since Gahlenbeck and Bartels (1970) did not provide $P_{\text{O}_2(\text{w})}$ values for which due allowance must be made (Fig. 1).

As increasing temperature leads to increased metabolic demand in poikilotherms, the value of $13.8\mu\text{l O}_2\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ at 30°C ($P_{\text{O}_2(\text{w})} = 152\text{mmHg}$) for aquatic respiration is lower than might be expected, and doubtless reflects a switch to aerial respiration as we have shown at reducing $P_{\text{O}_2(\text{w})}$ (Fig. 2), (in our system,

animals could and did push through the supernatant paraffin-layer to gulp air). At any given $P_{\text{O}_2(\text{w})}$ the availability of dissolved oxygen will be lower at 30°C than at 20°C; however, because of problems inherent to closed-system respirometry ($P_{\text{O}_2(\text{w})}$ decreasing and $P_{\text{CO}_2(\text{w})}$ increasing with time), it is impossible to state with certainty that any switch to air-breathing at this temperature is mediated by aquatic hypoxia. In some air-breathing fish, aquatic hypercarbia will also lead to a switch to lung-breathing, presumably by creating conditions that are more favourable to aerial CO_2 -exchange (Hughes and Singh, 1971). However, during our study individual slopes for *A. mexicanum* showed no evidence of threshold effects, supporting the contention that if P_{CO_2} -mediated switching to lung-breathing does occur, the relevant response threshold was not attained during the experimental period. Although an aquatic temperature of 30°C is somewhat in excess of that recommended for captive maintenance of axolotls (British Herpetological Society Captive Breeding Committee, 1978), experimental animals showed no signs of distress or abnormality either during or after exposure to this temperature regime.

West and Burggren (1982) working on tadpoles of *Rana catesbeiana*, suggested that aquatic oxygen tension exerted an effect on gill and lung ventilation which interact so that a high lung ventilation frequency in hypoxic waters acts to suppress gill ventilation cycles. This is consistent with our present observations on *A. mexicanum*, that seem to show that air-breathing increased and gill ventilation decreased under hypoxic conditions. At some as yet undefined threshold, severe environmental hypoxia will act to deplete systemic venous blood arriving at the gills. Furthermore, at low ambient oxygen tensions gill ventilation will become decreasingly efficient energetically, as the amount of work done increases in direct proportion to the decrease in the availability of dissolved oxygen. In paedomorphs of *A. tigrinum*, Malvin and Heisler (1988) have shown that an increase in the fraction of cardiac output flowing to the lungs and a decrease in the fraction to the gills accompanies aquatic hypoxia, the latter having a protective function. This is consistent with the view that in general the gills of perennibranchiate urodeles are of limited use as oxygen exchangers, though they may be important in CO_2 -exchange and acid-base regulation owing to the higher permeability of CO_2 .

It would appear from these studies that the axolotl is quite well adapted to ecologically realistic levels of hypoxia that might arise due to changes in temperature or biological oxygen demand in its normal habitat of a shallow, warm-belt lake at an altitude in excess of 2200m. Bimodal respiratory adaptations are characteristic of aquatic vertebrates inhabiting waters subject to periodic hypoxia and hypercarbia (Dehadrai and Tripathi, 1976; Ultsch, 1976). Unfortunately, few details of the natural history and habitat of *A. mexicanum* have been published (see Griffiths and Thomas, 1989). Even more unfortunate is the fact that the sole surviving wild population of the Mexican axolotl, in one lake near Mexico City, appears vulnerable so that it may soon become impossible to study the axolotl in its normal habitat (Griffiths and Thomas, 1989).

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