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DEVELOPMENTAL CHANGES IN OXYGEN CONSUMPTION REGULATION IN LARVAE OF THE SOUTH AFRICAN CLAWED FROG *XENOPUS LAEVIS*

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Summary

Well-developed larval *Xenopus laevis* (NF stages 58–66) are oxygen regulators, at least during mild hypoxia. When and how they change from oxygen conformers (the presumed condition of the fertilized egg) to oxygen regulators is unknown. Also unknown is how anaerobic metabolic capabilities change during development, especially in response to acute hypoxia, and to what extent, if any, anaerobiosis is used to supplement aerobic metabolism. Consequently, we have investigated resting rates of oxygen consumption ($\dot{M}_{O_2}$) and concentrations of whole-body lactate (lactic acid) during development in normoxia and in response to acute hypoxia in *Xenopus laevis*. $\dot{M}_{O_2}$ increased in an episodic, non-linear fashion during development. Resting, normoxic $\dot{M}_{O_2}$ increased about tenfold (to approximately 0.20 μmol g^{-1} h^{-1}) between NF stages 1–39 and 40–44, and then another tenfold between NF stages 45–48 and 49–51 (to approximately 2.0 μmol g^{-1} h^{-1}), remaining at about 2 μmol g^{-1} h^{-1} for the remainder of larval development. $\dot{M}_{O_2}$ reached its highest level in newly metamorphosed frogs (nearly 4 μmol g^{-1} h^{-1}), before decreasing to about 1.0 μmol g^{-1} h^{-1} in large adults. *X. laevis* embryos and larvae up to NF stage 54–57 were oxygen conformers when exposed to variable levels of acute hypoxia. The only exception was NF stage 45–48 (external gills present yet body mass still very small), which showed some capability of oxygen regulation. All larvae older than stage 54–57 and adults were oxygen regulators and had the lowest values of P_{crit} (the oxygen partial pressure at which $\dot{M}_{O_2}$ begins to decline). Whole-body lactate concentration in normoxia was about 1 μmol g^{-1} for all larval groups, rising to about 12 μmol g^{-1} in adults. Concentrations of lactic acid in NF stages 1–51 were unaffected by even severe ambient hypoxia. However, whole-body lactate levels in NF stages 52–66 increased in response to severe hypoxia, indicating that some anaerobic metabolism was being used to supplement diminishing aerobic metabolism. The largest increases in concentration of lactate occurred in late larvae and adults.

Key words: *Xenopus laevis*, oxygen consumption, oxygen regulation, development, frog.

Introduction

The oxygen consumption rate of adult South African clawed frogs (*Xenopus laevis*), as well as the resting and active rates of oxygen consumption of advanced larvae, are well known (see Burggren, 1984; Feder, 1981; Burggren and Just, 1992; Gatten *et al.* 1992, for reviews of the extensive literature). Well-developed larval *X. laevis* (NF stages 58–66) appear to be oxygen regulators, at least during mild hypoxia, and increase gill and lung ventilation rate and volume in response to hypoxic exposure (see Feder and Wassersug, 1984; Wassersug and Feder, 1983). However, the metabolic physiology of embryos and early larval stages of *Xenopus laevis* have rarely been investigated. Because a systematic investigation of metabolic responses to hypoxia is lacking, when and how they change from oxygen conformers (the hypothesized condition of the fertilized egg and youngest embryos) to oxygen regulators has not been determined to our knowledge. During hypoxia, anuran larvae, including those of *X. laevis*, accumulate anaerobic end products (primarily lactate), indicating the presence of significant anaerobic metabolism (Armentrout and Rose, 1971; Bennett and Licht, 1974; D'Eon *et al.* 1978; Gatz and Piiper, 1979; Feder, 1981, 1983; Feder and Wassersug, 1984). Also unknown for *X. laevis*, however, is how anaerobic metabolic capabilities change during development, especially in response to acute hypoxia, and to what extent anaerobiosis is used to supplement aerobic metabolism when oxygen is limiting. The issue of how all developmental stages (as opposed to just advanced larvae or adults) meet a hypoxic challenge has ecological as well as physiological relevance, for larval *X. laevis* are found in habitats ranging from permanently flowing, air-saturated water to stagnant water in buffalo wallows and temporary ponds with very low values of ambient P_{O_2} (Feder and Wassersug, 1984; Nieuwkoop and Faber, 1967).

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Given a lack of information on how ontogeny influences the basic metabolic responses to hypoxia in *X. laevis* (or, indeed, other anuran larvae), we consequently have investigated the changes in resting rates of oxygen consumption during development in normoxia and in response to acute hypoxia. Developmental changes in the value of P_{crit}, the critical P_{O_2} below which oxygen uptake begins to fall with decreasing P_{O_2}, have been determined, as have the corresponding changes in lactate production that signify anaerobic metabolism.

Materials and methods

Animals

Adult *Xenopus laevis* and their larvae and eggs were primarily acquired from adults bred at the University of Nevada, Las Vegas. These breeders, as well as some additional eggs and larvae, were obtained from commercial breeders (NASCO Scientific, Fort Atkinson, WI, USA) and XENOPUS I (Ann Arbor, MI, USA). Preliminary experiments comparing populations from different sources indicated no significant differences in developmental changes in metabolism or the metabolic responses to hypoxia. All animals were kept under a constant 12 h:12 h light:dark cycle at 20±1.0 °C and fed NASCO Frog Brittle frog food three times per week. All measurements were performed on animals that had not been fed for a minimum of 3 days in order to avoid measurement during a period of increased metabolic rate due to specific dynamic action.

Stage 1–66 tadpoles, generated in our laboratory by breeding adults, were kept at a concentration of about three larvae per liter of water in large (273–340 l) aquaria prior to experiments. One month post-metamorphic froglets were produced by placing 40 larvae (stage 66, see below) in an aquarium (340 l) and allowing them to complete metamorphosis. Fifteen adult *X. laevis* were obtained commercially for measurement of adult $\dot{M}_{O_2}$ values and were kept in groups of two or three in glass aquaria (90 l) prior to use. An additional 60 adults were used for measurements of whole-body lactate levels and six more adults for study of surfacing behavior.

Grouping of developmental stages

Experiments were conducted on *X. laevis* ranging in developmental stage from early embryos to adulthood, assessed according to the NF stage system of Nieuwkoop and Faber (1967). Rather than make measurements on each NF stage (some of which are based only on small anatomical changes), measurements were made on 11 different developmental groupings of animals, as follows: 1–39, 40–44, 45–48, 49–51, 52–53, 54–57, 58–60, 61–62, 63–66, froglets (1 month after metamorphosis) and sexually mature adults. Readers unfamiliar with the major anatomical and physiological changes associated with the NF stages corresponding to these developmental groupings are referred to the work of Nieuwkoop and Faber (1967) or Burggren and Just (1992).

Measurement of oxygen consumption

Total oxygen consumption (aquatic plus aerial) was determined by closed respirometry. Eggs and larvae were placed in respirometers consisting of 50 ml Erlenmeyer flasks containing 50 ml (minus the volume of the animals) of sterile tapwater and 2 ml of gas phase at the top of each respirometer. The opening of the respirometer flask was fitted with a rubber stopper with small intake and exhaust ports. A peristaltic pump pumping at a rate of 4 ml min^{-1} was used to draw gas from the top of the respirometer into a closed external gas-sampling loop of known volume. Respirometer gas entering this loop first passed into a 30 ml glass syringe containing a known but variable amount of gas. As gas samples were subsequently removed for O_2 analysis, the volume of gas in the syringe was reduced, preventing any pressure change within the respirometer system. After leaving the glass syringe, gas moved on to a sampling port from which gas passing through the loop could be sampled (see below). After leaving the sampling port, gas was returned to the respirometer, where it emptied out into the water at the bottom of the respirometer. The gentle bubbling of the water in the respirometer with returning gas ensured that the gas above the water in the respirometer was at complete equilibrium with the water in the respirometer, as was confirmed by preliminary measurements of P_{O_2} in both water and gas. The entire apparatus was thermostatted to 20 °C. Measurements on adults were made in the same fashion, except that the respirometer contained 1.0 l of water and 10 ml of gas. All chambers were covered with opaque material to minimize visual disturbance to the animals. All animals were allowed to acclimate to the respirometers for a minimum of 12 h before actual measurements were begun. The respirometers were open and equilibrated to room air during this acclimation period.

At the start of each experiment, a 1.0 ml gas sample was taken from the sampling port. Each gas sample produced a decrease of 1.0 ml in the volume of the glass syringe in the external sampling loop, which was noted and used in subsequent calculations. The percentage O_2 of this sample was measured with a Beckman model OM-11 oxygen analyzer. Gas samples (1.0 ml) were taken and analyzed approximately every 2 h. Measurements were continued until the gas reservoir in the syringe was emptied or until sharp decreases in the rate of incremental decline in respirometer O_2 began to signal impending asphyxiation.

The number of animals placed in each respirometer depended on their size and developmental stage (Table 1). When more than one animal was used in a single respirometer, the total oxygen consumption of the respirometer was divided by the total body mass of animals in the respirometer, generating a single $\dot{M}_{O_2}$ value for that development group (the equivalent of a value for one animal), and this was subsequently plotted and analyzed. Although consecutive measurements were performed on the same animals within the same respirometers, animals were not used again for measurements at other developmental stages.

Controls (respirometer blanks) were generated by measuring the $\dot{M}_{O_2}$ of respirometers that did not contain animals, but were exactly the same in every other way. This allowed for correction for microbial $\dot{M}_{O_2}$, although this was invariably less than 0.1 % of the $\dot{M}_{O_2}$ of the animals themselves.

The total rate of oxygen consumption (combination of aquatic and aerial) was calculated from the rate of decrease in oxygen concentration in the gas and water in the respirometer, using standard equations for closed respirometry. In all calculations, we assumed that 1 g of animal tissue occupied a volume of 1 ml in the respirometer. Rate of oxygen consumption ($\dot{M}_{O_2}$) was expressed as μmol O_2 g^{-1} h^{-1}.

Determination of P_{crit}

The technique of allowing P_{O_2} to be reduced by the animal's own metabolism produced a data set with $\dot{M}_{O_2}$ values matched with a wide range of P_{O_2} values on a continuum from 150 mmHg down to 20–40 mmHg. To facilitate analysis of these data, each measured P_{O_2} value and its associated $\dot{M}_{O_2}$ was placed in a 'P_{O_2} bin'. For example, P_{O_2} values between 46 and 55 mmHg were assigned the P_{O_2} value of 50 mmHg, P_{O_2} values between 96 and 105 mmHg were assigned the P_{O_2} value of 100 mmHg, etc. Linear regressions were then performed on the raw $\dot{M}_{O_2}$ values (not the means) associated with each 'bin' value of P_{O_2}. Fig. 2 presents these linear regressions, as well as the mean values (± 1 S.E.M.) of $\dot{M}_{O_2}$ for each P_{O_2} bin.

The least-squares regression method of Yeager and Ultsch (1989) was used to determine the P_{crit} for each of the developmental groups studied. When one regression fitted the data better than two regressions, the animals in that group were declared to be O_2 conformers with a P_{crit} at 150 mmHg. If two linear regression lines best fitted the data according to Yeager and Ultsch's (1989) procedure, the point at which the two regressions met was defined as the P_{crit}. Least-squares linear regressions were produced using Sigmastat statistical software from Jandel Corporation (San Rafael, CA, USA) on a Gateway 486DX computer.

Whole-body lactate levels

Animals to be assayed for lactate were kept in net enclosures in aquaria. The P_{O_2} of the water and gas above the water was varied using a Cameron mass flow controller. Following an overnight acclimation period in normoxia, animals were exposed to a specific P_{O_2} for a 2 h period and then removed for whole-body lactate analysis. The net enclosure was swiftly lifted from the aquarium and the animal dropped into liquid nitrogen – a procedure requiring 2–3 s. The solidly frozen animal was then put in a tissue grinder (larvae) or blender (adults) to produce a whole-body tissue slurry for lactate analysis.

Assays for whole-body lactate concentration were performed using Sigma Chemicals Corporation kit no. 826UV. Whole-body lactate concentrations are reported in μmoles of lactate per gram body tissue.

The number of tissue samples analyzed for each hypoxic exposure and the total number of animals pooled to form each sample are shown in Table 1.

Occurrences of surfacing

Surfacing in larval and adult *X. laevis* is usually for the purpose of lung ventilation. We measured surfacing frequency

Table 1. *Number of respirometer runs per* P_{O_2} *level and number of animals used per respirometer for* $\dot{M}_{O_2}$ *measurements, and numbers of tissue samples used for measurements of whole-body lactate concentration*

	$\dot{M}_{O_2}$ measurements		Lactic acid measurements	
Developmental stage	Number of separate respirometer measurements at each P_{O_2} level*	Number of animals used in each respirometer	Number of tissue samples at each P_{O_2} level†	Number of animals pooled for each tissue sample at each P_{O_2} level
NF 1–39	77, 36, 27, 31, 30, 32, 23, 23, 20	55–405	6	90–120
NF 40–44	22, 14, 24, 13, 24, 19, 20, 9, 3	50–162	10	150–200
NF 45–48	40, 17, 18, 13, 19, 10, 14, 10, 6	10–50	10	150–200
NF 49–51	33, 15, 11, 13, 15, 11, 12, 16	10–50	10	40
NF 52–53	27, 11, 12, 11, 13, 14, 11, 6, 3	1–10	10	10
NF 54–57	54, 25, 10, 11, 18, 26, 11, 7, 2	1–5	10	10
NF 58–60	31, 13, 15, 14, 8, 14, 13, 8	1-5	10	10
NF 61–62	16, 15, 11, 12, 12, 7, 9, 11, 10	1–5	10	10
NF 63–66	23, 12, 11, 15, 12, 19, 17, 16, 6	1–5	10	10
Froglets	52, 35, 17, 9, 13, 24, 12, 30, 10	1	6	6
Adults	12, 13, 13, 9, 18, 14, 18, 13, 8, 7, 6, 3	1	6	6

*Number of animals at each P_{O_2} level, beginning with a P_{O_2} of 150 mmHg and progressing to the most hypoxic level; value equivalent to the *N* value used for statistical analyses of $\dot{M}_{O_2}$.

†Equivalent to the *N* value used for statistical analyses of lactate.

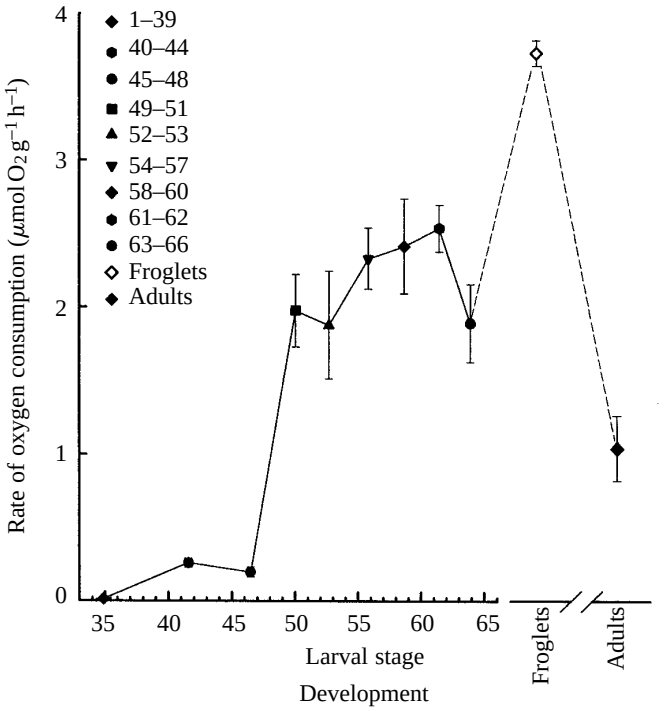

Fig. 1. Changes in $\dot{M}_{O_2}$ in response to development in normoxia. Each point represents the average normoxic $\dot{M}_{O_2}$ at 20 °C for each developmental group. Error bars indicate ±1 standard error of the mean. Error bars are smaller than symbols in some cases. Values of *N* for this and all subsequent figures are given in Table 1. Developmental stages according to Nieuwkoop and Faber (1967). Dashed line indicates a significant gap in the developmental continuum.

in NF stage 63–66, in 1 month post-metamorphic froglets and in adult frogs, as a function of the degree of hypoxia. Surfacing frequency was measured by placing individual animals in a chamber made from an upright 76 cm tall piece of black plastic pipe. The chamber was filled with water except for an 8 cm tall, 40 ml gas space at the top of the chamber from which the animal breathed. A lift tube within the chamber mixed and aerated the water, which was thermostatted at 20±0.1 °C. A pair of holes was drilled at opposing points in the wall of the chamber 15 cm from its top. A photoelectric sensor was then glued in one hole and a light-emitting diode was glued into the other hole. The light sensor was then connected to a physiograph. The movement of an older larva or frog towards the surface for gas breathing interrupted the light beam to the sensor, producing pen movement on the chart recorder. This pen recording was then used to determine the rate of surfacing occurrences. Animals in the chamber were exposed to normoxia and then to the same levels of hypoxia used in the previously described experiments. The water and the gas above it always had the same P_{O_2}. Hypoxia was produced by a Cameron mass flow controller.

The effect of hypoxia on surfacing frequency was tested for significance using an analysis of variance (ANOVA) for repeated measures.

Results

Developmental changes in $\dot{M}_{O_2}$ *during normoxia*

Resting, normoxic $\dot{M}_{O_2}$ increased in an episodic, non-linear fashion during development (Fig. 1). Although the absolute levels of $\dot{M}_{O_2}$ were low early in development, these values increased approximately tenfold by NF stage 40–44 (to approximately 0.20 μmol g^{-1} h^{-1}) and then again between groups NF stage 45–48 and NF stage 49–51 (to approximately 2.0 μmol g^{-1} h^{-1}). $\dot{M}_{O_2}$ then remained at about 2.0 μmol g^{-1} h^{-1} until it increased again in 1 month post-metamorphic froglets to about 4.0 μmol g^{-1} h^{-1}. Finally, $\dot{M}_{O_2}$ fell to only about 1.0 μmol g^{-1} h^{-1} for the large adults.

Developmental changes in $\dot{M}_{O_2}$ *during hypoxia*

All developmental stages showed significant $\dot{M}_{O_2}$ values even in severe hypoxia (ambient P_{O_2} about 35 mmHg). However, there was great variation in the extent to which $\dot{M}_{O_2}$ declined from normoxic levels during severe hypoxia. The $\dot{M}_{O_2}$ ranges (highest to lowest) in the following discussion refer to the $\dot{M}_{O_2}$ during normoxia and at the lowest ambient P_{O_2} in which oxygen removal from the environment within the respirometer could be measured.

The $\dot{M}_{O_2}$ values of NF stage 1–39 ranged from 0.006 to 0.014 μmol g^{-1} h^{-1} (Fig. 2A). These animals were clearly oxygen conformers, unable to maintain normoxic levels of $\dot{M}_{O_2}$ even during mild hypoxia (Fig. 2A). The $\dot{M}_{O_2}$ of NF stage 40–44 ranged from 0.013 to 0.256 μmol g^{-1} h^{-1} (Fig. 2B). This was the youngest group that showed any evidence of oxygen regulation, with a P_{crit} of approximately 71 mmHg.

The $\dot{M}_{O_2}$ values of NF stage 45–48, NF stage 49–51, NF stage 52–53 and NF stage 54–57 ranged from 0.010 to 0.198, from 0.028 to 1.978, from 0.049 to 1.879 and from 0.379 to 2.457 μmol g^{-1} h^{-1}, respectively (Fig. 2B–D). Unlike the immediately preceding developmental group, NF stage 45–57 larvae were all oxygen conformers.

NF stage 58–60, NF stage 61–62 and NF stage 63–66 had $\dot{M}_{O_2}$ values ranging from 0.0793 to 2.4168, from 0.3193 to 2.5152 and from 0.6206 to 1.8914 μmol g^{-1} h^{-1} (Fig. 2D,E). All of these developmental groups were oxygen regulators, with P_{crit} values of 78, 76 and 86 mmHg, respectively.

Post-metamorphic froglets and adult frogs had $\dot{M}_{O_2}$ values ranging from 0.230 to 3.850 and from 0.344 to 1.085 μmol g^{-1} h^{-1}, respectively (Fig. 2F). Both froglets and adults were oxygen regulators, with P_{crit} values of 65 and 30 mmHg, respectively.

To summarize, animals of all developmental stages showed some decrease in $\dot{M}_{O_2}$ as ambient P_{O_2} decreased, as indicated in Fig. 3. Normoxic $\dot{M}_{O_2}$ increased during development, peaking with metamorphosis, and then declining again in adults. Overall changes in P_{crit} as a function of development are shown in Fig. 4. The ability to regulate oxygen first appeared in NF stage 40–44, but was not entrenched until NF stage 58 and beyond, when P_{crit} occurred at 85 mmHg or below. P_{crit} generally decreased as development progressed from NF stage 58 to adulthood.

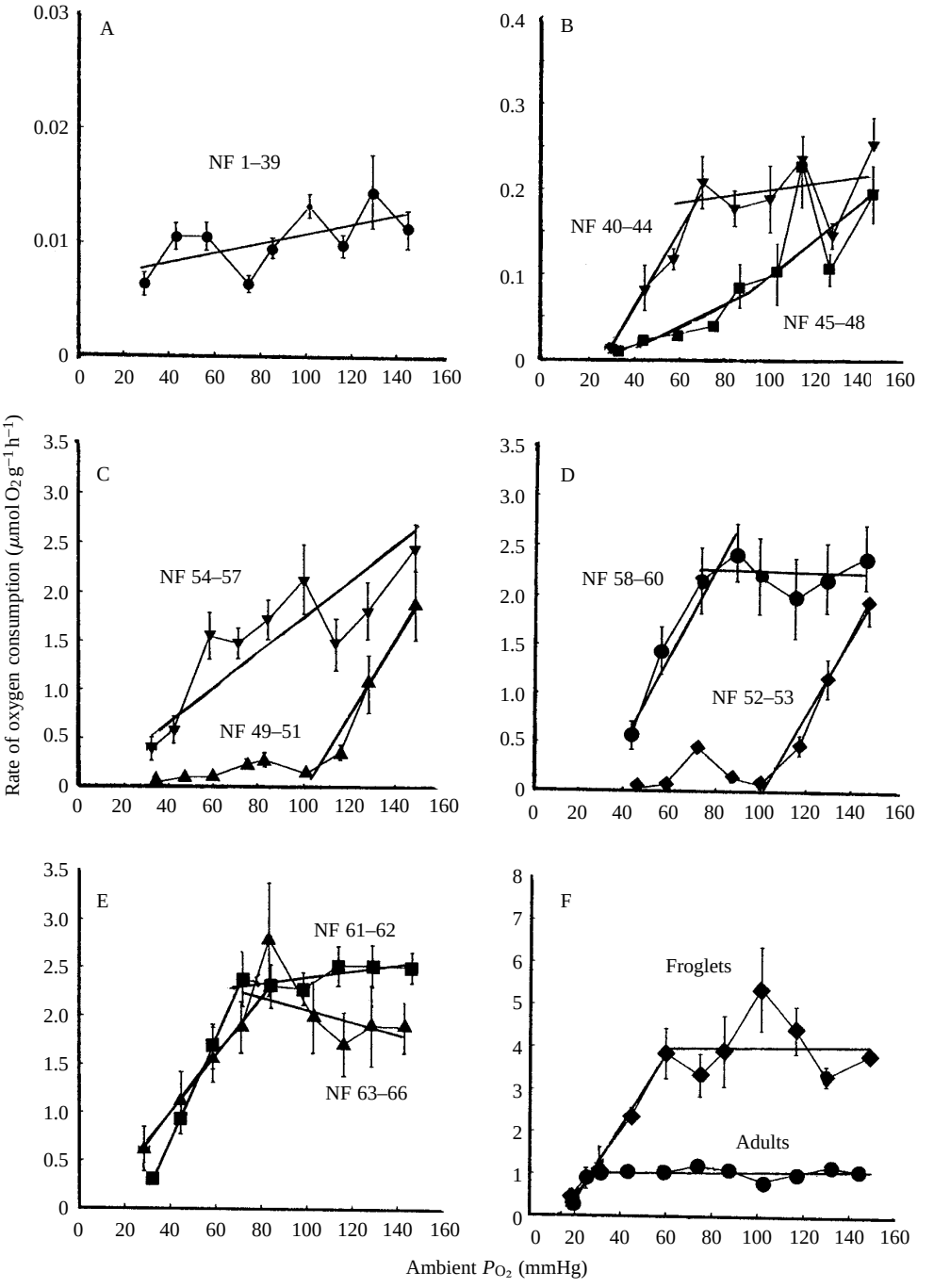

Fig. 2. $\dot{M}_{O_2}$ as a function of ambient P_{O_2} in NF stage 1–66 larvae, 1 month post-metamorphic froglets and adult frogs. Each point represents the mean $\dot{M}_{O_2}$ (±1S.E.M.) at each P_{O_2} level. Error bars are smaller than symbols in most plots. Note that the scale on the ordinate differs from panel to panel. P_{crit} values, where appropriate, are provided in Fig. 4. Values of N are given in Table 1. Regressions in this figure, as well as those in Fig. 5, are calculated after the method of Yeager and Ultsch (1989); see text for details.

Whole-body lactate concentrations during normoxia

Mean whole-body lactate concentration during normoxia ranged from a low of 0.9±0.1 μmol g^{-1} in stage 49–51 larvae to a high of 2.3±0.2 μmol g^{-1} in stage 45–48 larvae. 1 month post-metamorphic froglets had a comparable mean whole-body lactate concentration during normoxia of 1.6±0.2 μmol g^{-1}. However, adult frogs had a considerably increased mean whole-body lactate concentration during normoxia of 12.4±1.4 μmol g^{-1}.

Whole-body lactate concentrations during hypoxia

The following ranges in whole-body lactate concentrations correspond to levels measured during normoxia up to those in the most hypoxic conditions that the group experienced, as indicated in Fig. 5A–F. Whole-body lactate levels in individuals of NF stage 1–39, NF stage 40–44 and NF stage 45–48 had ranges of 0.7–1.4, 1.2–2.0 and 1.0–2.9 μmol g^{-1}, respectively (Fig. 5A,B). Whole-body lactate concentrations for NF stage 49–51, NF stage 52–53 and NF stage 54–57 were 0.6–1.1, 0.8–1.9 and 1.1–4.0 μmol g^{-1}, respectively (Fig. 5C,D). NF stage 58–60, NF stage 61–62 and NF stage 63–66 had whole-body lactate levels of 0.6–1.8, 1.4–5.2 and 1.2–3.7 μmol g^{-1}, respectively (Fig. 5D,E). 1 month post-metamorphic froglets and adult frogs had whole-body lactate

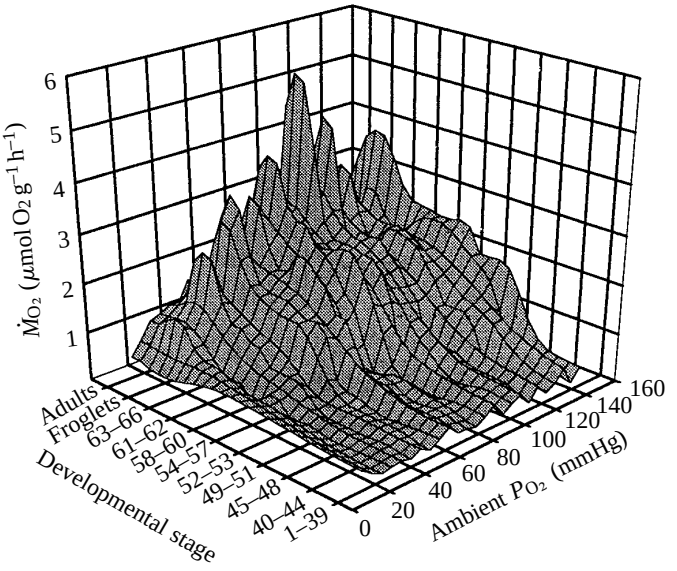

Fig. 3. Summary of the changes in $\dot{M}_{O_2}$ as a function of ambient P_{O_2} and development in *Xenopus laevis*. Ambient P_{O_2} ranged from 150 to 20 mmHg.

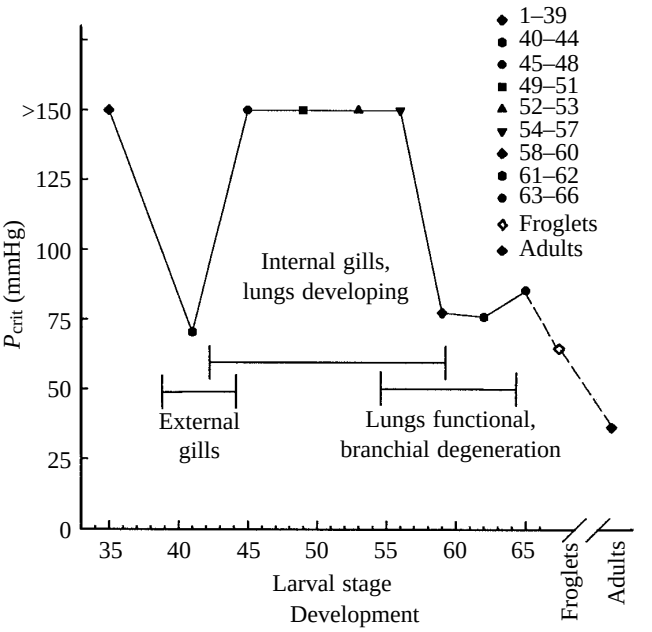

Fig. 4. Changes in P_{crit} as a function of development. Horizontal bars indicate the developmental stages through which each defined respiratory structure is developing. Oxygen conformers have a P_{crit} at or above 150 mmHg indicated by >150. Values of P_{crit} were obtained from Fig. 2.

levels of 2.0–5.0 and 8.0–27.0 μmol g^{-1}, respectively (Fig. 5F).

Fig. 6 summarizes the interactions of P_{O_2}, whole-body lactate concentration and development. Whole-body lactate concentrations in response to severe hypoxia increased about midway through larval development. Until stage 51, whole-body lactate concentrations did not increase significantly in response to any level of hypoxia. From stage 52 onwards, whole-body lactate concentrations during severe hypoxia were significantly higher than normoxic levels.

To facilitate comparison and to indicate how lactate production during hypoxia changes with development, whole-body lactate concentrations at a P_{O_2} 15 mmHg below the P_{crit} of each developmental group are plotted in Fig. 7. Whole-body lactate levels just below P_{crit} remained fairly constant for all the larval developmental groups through metamorphosis at approximately 1 μmol g^{-1}. Whole-body lactate concentrations for 1 month post-metamorphic froglets and adult frogs increased to about 10 and 25 μmol g^{-1} respectively at 15 mmHg below P_{crit} (Fig. 7).

Surfacing frequency

Surfacing frequency for each of the three developmental groups of animals studied showed no significant change from control values at normoxia until after ambient P_{O_2} had fallen below that group's P_{crit}. After ambient P_{O_2} had been reduced below the P_{crit}, surfacing frequency increased significantly (Fig. 8). NF stage 63–66 larvae surfaced approximately five times per hour when the ambient P_{O_2} was above the P_{crit}, increasing to approximately 20 times per hour when exposed to a P_{O_2} around 30 mmHg. Post-metamorphic froglets and adult frogs surfaced approximately 15–20 times per hour when exposed to an ambient P_{O_2} between 150 and 85 mmHg. The number of surfacing occurrences increased linearly from 20–30 as P_{O_2} fell from 80–40 mmHg. Death of individual froglets began to occur at an ambient P_{O_2} below 40 mmHg. Unlike froglets, adult frogs dramatically increased surfacing frequency to approximately 65 times per hour when exposed to a P_{O_2} of 20 mmHg.

Discussion

Developmental changes during normoxic $\dot{M}_{O_2}$

Although the general trend during larval development was for an increase in $\dot{M}_{O_2}$, there were several discontinuities in this pattern. For example, although NF stage 1–39 and NF stage 40–44 have approximately the same mass and body size, and are only hours apart in development, the $\dot{M}_{O_2}$ of stage 40–44 was approximately ten times that of the earlier developmental group (Figs 1, 2). Interpretation of these and other profound changes in $\dot{M}_{O_2}$ during development are confounded when the data are expressed on a mass-specific basis, because individuals change in composition as well as size when they develop. In eggs, the vast majority of mass consists of 'non-living', metabolically inert lipids stored in yolk cells within the egg. As the embryo matures, the lipids are incorporated into living, oxygen-consuming tissue. When the young larvae are ready to hatch, excess yolk cells are drawn into the body of the larvae to be metabolized later. Many yolk platelets are present in newly hatched larvae, and they remain in the larvae up to

stage 39 (Nieuwkoop and Faber, 1967). Therefore, accurate measurement of the mass of metabolically active tissue is very difficult in the early developmental stages. Expressing mass as embryo plus yolk considerably underestimates the mass-specific $\dot{M}_{O_2}$ and may erroneously suggest the greatly accelerating aerobic metabolism accompanying yolk depletion which has frequently been reported for anurans (Bialaszewicz and Bledowski, 1915; Parnas and Kaskinska, 1921; Brachet, 1934; Wills, 1936; Hopkins and Handford, 1943; Gregg, 1960). Once yolk absorption has occurred, comparisons of $\dot{M}_{O_2}$ are more meaningful.

Our values for resting $\dot{M}_{O_2}$ during normoxia in midlarval *X. laevis* are comparable with previously reported values. For example, Feder (1981) reported a fairly constant $\dot{V}_{O_2}$ (converted by us to $\dot{M}_{O_2}$) of 2.25–4.5 μmol g^{-1} h^{-1} for stage 49–66 larvae, while Fletcher and Myant (1959) reported an $\dot{M}_{O_2}$ of 3.7–5.5 μmol g^{-1} h^{-1} for the same stages of larval *X. laevis* during activity. The reason for the large rise in $\dot{M}_{O_2}$ between stages 46 and 48 in the present study is unclear. This is the time at which the lungs develop and air-breathing begins, and the rise in $\dot{M}_{O_2}$ during normoxia could reflect a combination of an increased oxygen demand at the tissue level coupled with an increase in the larva's ability to acquire oxygen from its environment. As noted below, however, it is

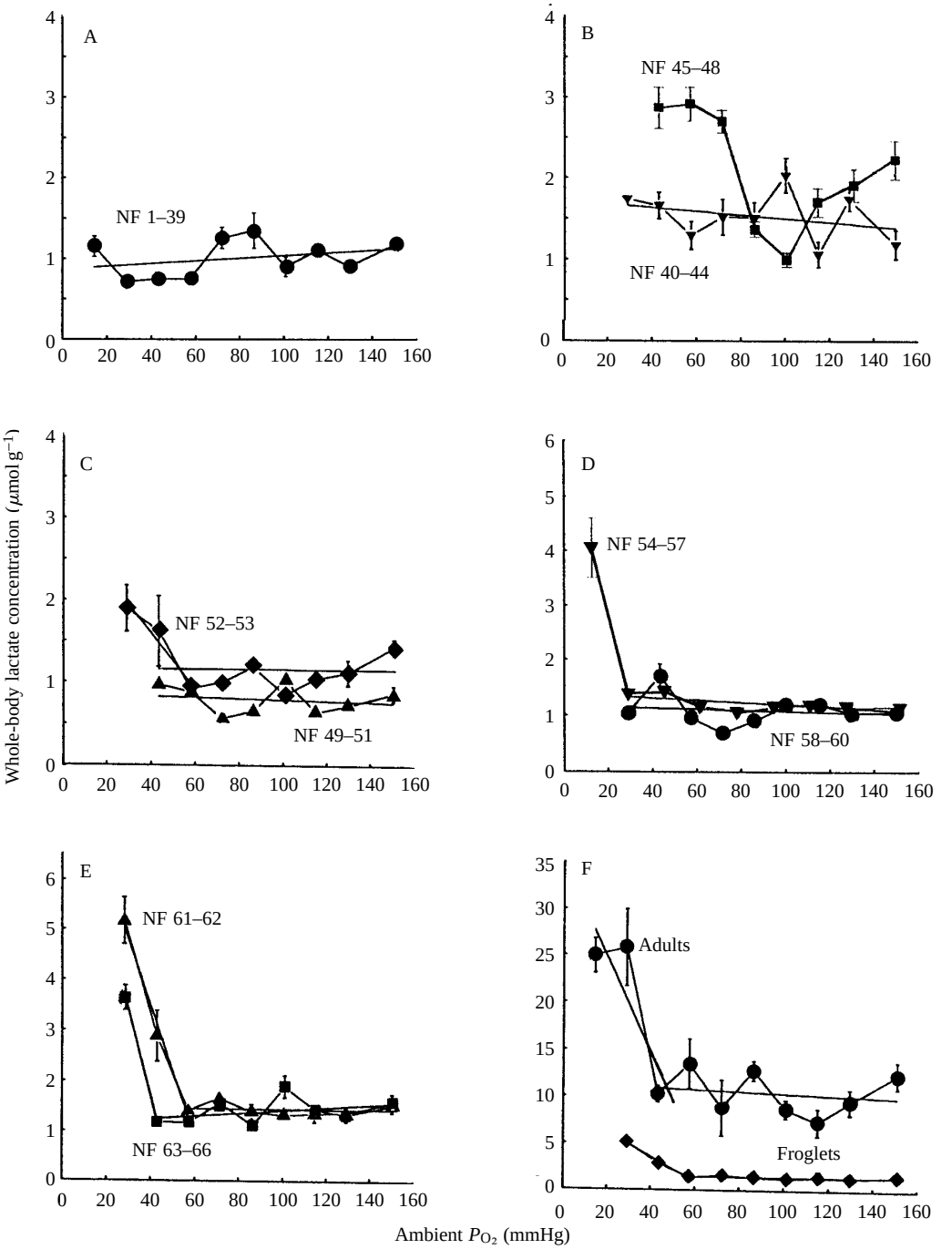

Fig. 5. Whole-body lactate concentrations as a function of ambient P_{O_2} in stage 1–66 larvae, 1 month post-metamorphic froglets and adult frogs. Each point represents the mean (±1S.E.M.) whole-body lactate concentration at each P_{O_2} exposure. Note that the scale on the ordinate differs from panel to panel. Values of *N* are given in Table 1.

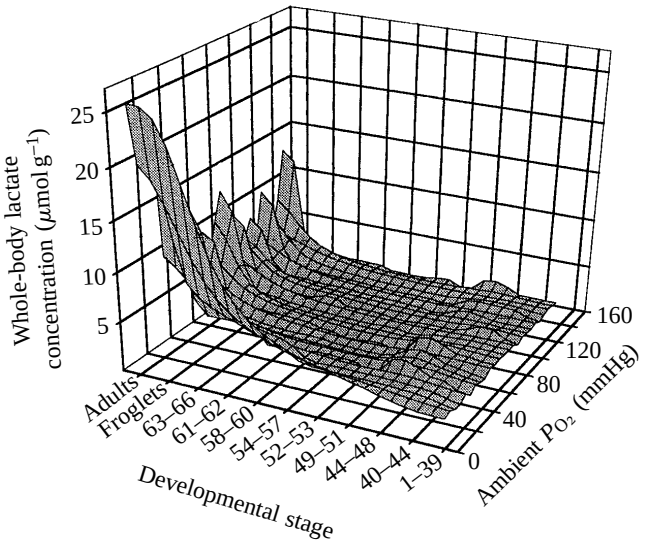

Fig. 6. Summary of the effect of development and hypoxia on average whole-body lactate concentrations in *Xenopus laevis*. Ambient P_{O_2} ranged from 20 to 150 mmHg.

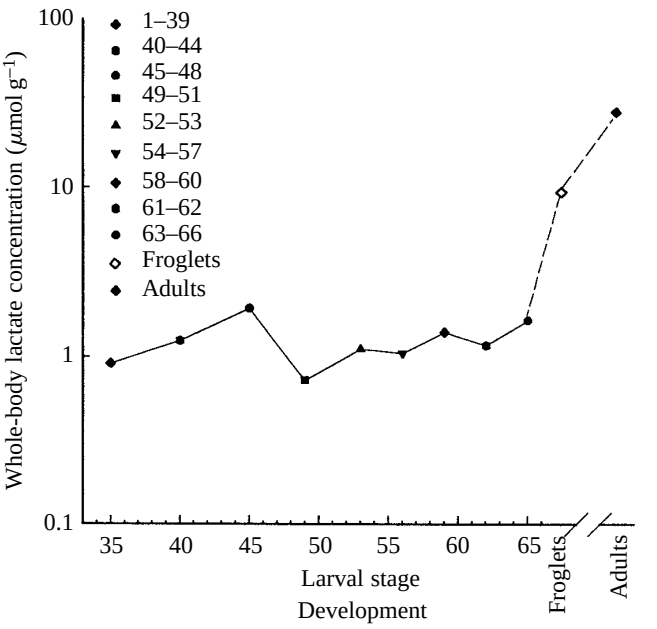

Fig. 7. Whole-body lactate concentrations (plotted on a logarithmic scale) at a P_{O_2} 15 mmHg below the P_{crit} as a function of developmental stage in *Xenopus laevis*. Values are taken from the results given in Fig. 5.

also at these stages that the larvae revert to oxygen conformation, suggesting that the combination of all available gas exchange organs at this point in development is inadequate to garner sufficient oxygen to support oxygen consumption during hypoxia.

$\dot{M}_{O_2}$ during normoxia stayed relatively constant throughout middle and late larval development, as the lungs become fully functional and the internal, ventilated gills degenerate in preparation for metamorphosis. $\dot{M}_{O_2}$ increased significantly after metamorphosis and the completion of lung development (Figs 1, 2). This increase could have occurred if incomplete lung development was limiting oxygen uptake, but *X. laevis* and other anuran larvae are quite capable of high levels of oxygen uptake through their skin (Feder and Burggren, 1985).

$\dot{M}_{O_2}$ was highest in froglets, perhaps related to the tendency for increased locomotor activity in this developmental stage. $\dot{M}_{O_2}$ was lower in adults. Resting $\dot{M}_{O_2}$ for adults in the present study (1.1 μmol g^{-1} h^{-1}) is also in agreement with reported values of 1.2–3.8 μmol g^{-1} h^{-1} for adult *X. laevis* at similar temperatures (see Gatten *et al.* 1992, for a review).

The developmental transition from oxygen conformation to oxygen regulation

Since normoxic $\dot{M}_{O_2}$ measurements of each of the different developmental groups of animals in these experiments agree with previously reported values, we assume that the hypoxic $\dot{M}_{O_2}$ values we have measured are equally accurate since they employed the same experimental protocol, methods and apparatus.

NF stage 1–39 larvae were oxygen conformers, but their $\dot{M}_{O_2}$ even during normoxia was so low that the incremental decline in $\dot{M}_{O_2}$ with increasingly severe hypoxia was not large. This suggests that these animals are not highly dependent on aerobic metabolism, as has been reported by prior investigations of amphibian embryos (for reviews, see Burggren, 1984; Feder, 1984). Unfavorable conditions cause embryos to reduce or even stop cell division and to go into a dormant period (Detwiller and Copenhaver, 1940; Gregg, 1960; Rose *et al.* 1971; Weigmann and Altig, 1975). Consequently, the observation that early embryos (prior to stage 39) in the present study continued to progress through development at a normal rate while in hypoxia within their respirometers further supports the hypothesis that anaerobic metabolism is able to provide for their energetic needs. It is noteworthy, however, that we could detect no significant increase in whole-body lactate levels during hypoxia below the P_{crit} in these earliest stages (note the logarithmic scale in Fig. 7). This probably reflects the overall low level of metabolism, either aerobic or anaerobic, and the ensuing low overall level of lactate in the tissues of these earliest developmental stages.

NF stage 40–44 larvae were fairly effective oxygen regulators, maintaining $\dot{M}_{O_2}$ at normoxic levels until ambient P_{O_2} fell below approximately 70 mmHg. The development of external gills in these stage 40–44 larvae, along with a large surface-area-to-volume ratio and small body mass (with attendant short gas diffusion distances), may account for why these very young larvae can regulate their $\dot{M}_{O_2}$ in response to mild decreases in ambient P_{O_2} (Figs 2, 4).

Coincident with the loss of external gills at stage 45, larvae in NF stages 45–48 returned to oxygen conformation, a pattern maintained through development to about stage 57 (Fig. 4).

The decline in $\dot{M}_{O_2}$ is particularly sharp during moderate hypoxia, quickly falling to very low levels below a P_{O_2} of about 100 mmHg. This reversion to oxygen conformation seems paradoxical, since animals at these developmental stages have added pulmonary gas exchange to their respiratory repertoire. However, even though the lungs first start to form in the early larval stages, they may not provide the full respiratory gas-exchange potential evident later in development. The lung walls initially consist only of a flat, squamous epithelium and so are still much less complex than the lungs of an adult. Lung ventilation at this stage in *X. laevis* may be as much for buoyancy regulation as for gas exchange (van Bergeijk, 1959). Not only might the lungs of early larvae be ineffective organs for oxygen uptake, but the internal gills of early larvae may be as important or even more important in filter feeding than in gas exchange, so the internal gills may initially not be as effective at removing oxygen from the water as the external gills. The buccopharynx of larval *X. laevis* has only poor gas-exchange capabilities (Czopek, 1955). All of these developmental changes may contribute to our observation that larval *X. laevis* between NF stages 45 and 57 are oxygen conformers.

By NF stage 54, the rate of decrease in aerobic metabolic rate in response to mild hypoxia is less drastic than in younger larvae. There are wide variations in hypoxic $\dot{M}_{O_2}$ between larvae of the same developmental stage, which implies that some may be oxygen regulating while others at this stage are still oxygen conformers. That is, stage 54–57 seems to be the transition period from oxygen conformation to oxygen regulation.

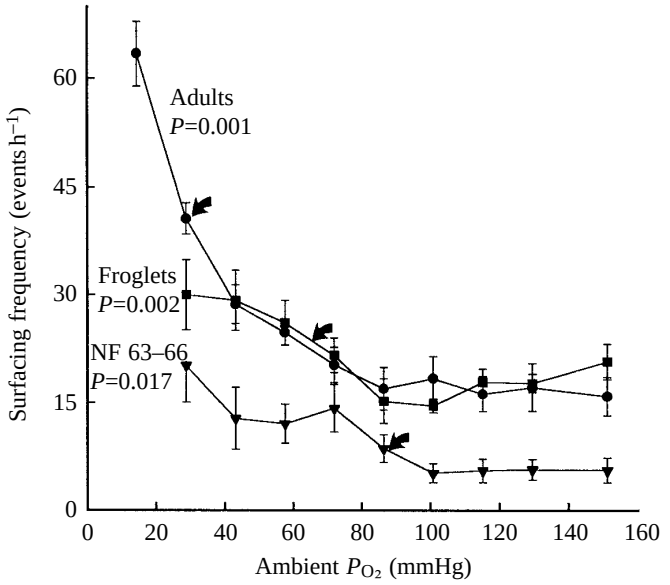

Fig. 8. Changes in surfacing frequency (occurrences per hour) measured during 2 h of hypoxia in stage 63–66 tadpoles, 1 month post-metamorphic froglets and adult *Xenopus laevis*. P_{O_2} ranged from 150 to 15 mmHg. Measurements were made on six individuals at each of the indicated developmental stages. Values are means ± 1 S.E.M. P values for analysis of variance assessing the effect of hypoxia on surfacing frequency are indicated for each developmental stage. Arrows indicate P_{crit} for each developmental stage.

NF stage 58 and older are oxygen regulators (Figs 2–4). After this stage, the lungs have gained a somewhat honeycomb-like compartmentalization with much greater surface area than in early larval stages. This increased structural development of the lungs may be the primary factor allowing these animals to regulate oxygen.

The hypoxic $\dot{M}_{O_2}$ for 1 month post-metamorphic froglets was consistently higher than that of all other developmental groups until ambient P_{O_2} was reduced to around 30 mmHg (Fig. 2). At 30 mmHg, the $\dot{M}_{O_2}$ was approximately 1.0 μmol g^{-1} h^{-1}, but this proved to be an insufficient aerobic metabolic rate since mortality was 75 %. Adult *X. laevis* could maintain their normal $\dot{M}_{O_2}$ down to a P_{O_2} of 30 mmHg. This is in contradiction to a prior study performed by Hutchison and Miller (1979) on slightly smaller *X. laevis* (15–50 g in their study, compared with 20–70 g in ours), in which adults could not regulate oxygen uptake below a P_{O_2} of 90 mmHg at 25 °C or 120 mmHg at 15 °C. During their study, the aerial and aquatic portions of the ambient environment were isolated by a layer of mineral oil to separate aerial and cutaneous $\dot{M}_{O_2}$. Perhaps the less disruptive method of the present study for measuring only total $\dot{M}_{O_2}$ accounted for these differences.

Surfacing and hypoxia

Significant changes in surfacing frequency in response to hypoxia occurred during development (Fig. 8). All three developmental groups increased the frequency of surfacing in response to hypoxia only after the P_{crit} for $\dot{M}_{O_2}$ had been reached. However, stage 63–66 larvae consistently surfaced less often than both 1 month post-metamorphic froglets and adult frogs. This pattern held during hypoxic exposure, indicating that there are two distinctly different patterns of lung ventilation between pre-metamorphic and post-metamorphic animals, as emphasized by Burggren and Infantino (1994).

Whole-body lactic acid concentrations during normoxia

Glycolysis is the only significant anaerobic pathway available to exercising amphibians. Muscle glycogen is degraded and lactate accumulates, but concentrations of other metabolites such as pyruvate, succinate and alanine change very little (Bennett, 1978). Therefore, measurement of lactate is a useful indicator of the extent of anaerobic metabolism.

Whole-body lactate concentrations for *X. laevis* stage 1–66 and 1 month post-metamorphic froglets at rest during normoxia remained at approximately 1–2.5 μmol g^{-1} and show no consistent pattern of change with increasing development. These values are somewhat lower than those of 3.4–4.3 μmol g^{-1} reported in a previous study for these stages of *X. laevis* during normoxia at 25 °C (calculated from Feder and Wassersug, 1984). The whole-body lactate concentrations for adult frogs (12.5 μmol g^{-1}) during normoxia are on the high side of the reported lactate concentrations for adult *X.*

laevis of about 1.2–10.1 μmol g^{-1} (Miller and Camilliere, 1981; Hutchison and Miller, 1979; Putnam, 1979; Boutilier *et al.* 1986).

Whole-body lactate concentrations during hypoxia

There was apparently no Pasteur effect in NF stage 1–51 embryos and larvae. Whole-body lactate concentrations did not increase significantly in response to even severe hypoxia (Figs 5, 6). The larvae significantly reduced their aerobic metabolic rate, but apparently a large increase in anaerobic metabolic rate (as measured by lactate production) was not used to supplement the decrease in aerobic metabolic rate. Whole-body lactate concentration for stage 45–48 falls as ambient P_{O_2} decreases to 100 mmHg, but then starts to rise sharply as the P_{O_2} is decreased further (Fig. 5). This rise may be due to changes in the frequency of surfacing as the ambient P_{O_2} falls. Because swimming activity is fueled primarily by anaerobic metabolism (Hillman and Withers, 1981; Miller, 1983), the rise in whole-body lactate concentration below a P_{O_2} of 100 mmHg may result from an increased surfacing frequency to breathe air (which would be hypoxic according to the experimental design). Interestingly, the whole-body lactate concentrations of stage NF 49–51 during severe hypoxia are only slightly higher than during normoxia (Fig. 5). This suggests that anaerobic metabolism, as measured by whole-body lactate concentrations, may not be heavily involved in supplementing the aerobic metabolism of this particular developmental stage.

Beginning with NF stage 52, and through the rest of development, whole-body lactate concentrations were not elevated until ambient P_{O_2} fell below about 40–60 mmHg (Fig. 5). Below this level of P_{O_2}, lactate concentration increased sharply. It appears that stages 52–53 are the first stages in development where anaerobic metabolism, as measured by whole-body lactate level, can be evoked for minimal maintenance of body tissues during severe hypoxia. Overall, these data on lactate concentrations fit well with the general developmental trend towards oxygen regulation. That is, lactate concentration increases sharply only below the P_{crit} of a given developmental stage.

This study on developing *X. laevis* has shown a strong developmental effect on both the pattern of oxygen regulation and the use of anaerobiosis to supplement aerobic metabolism during hypoxia. Those developmental stages capable of oxygen regulation are also the stages with anatomical structures that would appear to be well-suited for gas exchange in that particular stage. Similarly, changes from oxygen conformation to oxygen regulation (or *vice versa*) appear to be correlated with changes in the structure or existence of external gills, internal gills, lungs and skin. Particularly intriguing is the observation that the transition from fully developed external gills to developing internal gills is associated with a sharp rise in P_{crit}. Future studies that specifically enhance or reduce the ability of particular organs to participate in gas exchange may provide new information about the physiological basis for oxygen regulation in *X. laevis*.

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