

THE EFFECTS OF AQUATIC OXYGEN
CONCENTRATION, BODY SIZE AND RESPIRATORY
BEHAVIOUR ON THE STAMINA OF OBLIGATE
AQUATIC (*BUFO AMERICANUS*) AND FACULTATIVE
AIR-BREATHING (*XENOPUS LAEVIS* AND *RANA*
BERLANDIERI) ANURAN LARVAE

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SUMMARY

Larvae of the anurans *Rana berlandieri* and *Xenopus laevis* have lungs and can breathe air as well as irrigate buccal and pharyngeal surfaces for aquatic respiration. Larvae of *Bufo americanus* lack lungs until just before metamorphosis and are obligately aquatic. We examined the relationship between the locomotor stamina (time to fatigue), aquatic oxygen concentration, body size, and respiratory behaviour of swimming larvae of these species, with the following results:

- (1) Stamina is size-dependent in all three species.
- (2) Aquatic hypoxia reduces stamina in larvae of all three species, but most conspicuously in *Bufo*.
- (3) Breathing air increases stamina in *Rana* larvae, especially in large animals and under aquatic hypoxia.
- (4) In contrast to *Rana* larvae, *Xenopus* larvae swimming in normoxic water undergo a reduction in stamina when allowed to breathe air. In hypoxic water, aerial respiration moderates the reduction in stamina seen in *Xenopus* larvae.
- (5) Branchial irrigation is associated with increased stamina in *Xenopus*, and is increased under hypoxia and at high swimming velocities. Respiratory demand, buoyancy and the drag associated with branchial irrigation all affect respiratory behaviour in *Xenopus* larvae.

The great amount of interspecific variation in the relationship between respiratory behaviour and stamina reveals the importance of measuring performance directly when attempting to interpret the functional significance of respiratory structures and behaviour.

INTRODUCTION

The physical differences between air and water as respiratory media are well known. Air has a much greater oxygen capacitance than water; it is less dense, less viscous, less resistant to diffusion and less frequently hypoxic (Dejours, 1976). Hence many aquatic vertebrates supplement branchial and cutaneous oxygen uptake by breathing air. Such animals must contend with diverse ecological and ergometric consequences of breathing air. A fish or amphibian that swims to the surface to breathe air is conspicuous and attracts both aerial and aquatic predators (Kramer & Graham, 1976; Drummond, 1980). Air-breathing may have substantial energetic costs (Kramer & McClure, 1981), which are sufficient to retard growth (Arunachalam, Vivekanandan & Pandian, 1976; Pandian & Vivekanandan, 1976). Also, carrying air under water in lungs or other organs may increase buoyancy and compromise locomotor performance, particularly in currents (Hora, 1930; Gee, 1977, 1981). However, not breathing air could affect stamina and resistance to hypoxia. Given this array of costs and benefits, it is difficult to predict the circumstances in which facultative air-breathers will increase their dependence upon aerial respiration.

Our approach has been to measure directly the effect that the use of aerial respiration has on one measure of performance, locomotor stamina, for anuran larvae. Also, we have characterized normal patterns of respiration in active animals in an attempt to reconcile these patterns with their presumed costs and benefits. We have studied three species. An important difference among the three species under study is that *Xenopus* larvae are far more buoyant than *Rana* or *Bufo* larvae; immobile *Xenopus* larvae float to the water's surface while immobile *Rana* and *Bufo* larvae sink to the bottom. In still water, *Xenopus* larvae hover midwater in a head down posture and scull with their tail filament to counterbalance the buoyancy of their lungs (Van Bergeijk, 1959). Larvae of *Rana berlandieri* and *Xenopus laevis* are facultative air breathers. Larvae of *Bufo americanus* lack functional lungs until just before metamorphosis and thus breathe water alone. Larvae of all three species are negatively rheotropic and will swim upstream against a current. We have taken advantage of this behaviour to compare the effects of several factors on locomotor performance: high *vs* low aquatic oxygen concentration, large *vs* small size and presence *vs* absence of functional lungs. Some additional data are presented on the relationship of aquatic respiratory rates to the stamina of *Xenopus* larvae.

Our results reveal extensive interspecific variation in the respiratory behaviour of tadpoles. In certain cases the patterns seen in one species are the reverse of patterns seen in another. Our work demonstrates the importance of actually measuring performance in assessing the importance of respiratory structures and behaviour, rather than indirectly inferring adaptive significance from presumed physiological correlates of performance such as metabolic rates.

MATERIALS AND METHODS

The techniques for the care, feeding and measurement of larvae have been described previously (Feder, 1981; Wassersug, Paul & Feder, 1981). Larvae we

staged according to Gosner (1960) and only premetamorphic larvae (<stage 40) were used in these experiments. Snout-vent length, total length and dry mass were recorded for each tadpole; however, since these variables were all highly intercorrelated we used dry mass (mg) as the primary size variable in our statistical analyses. An equation relating dry mass to wet mass for anuran larvae is given in Feder (1981). Size data in the text are presented as $\bar{x} \pm \text{s.d.}$ unless otherwise noted. All experiments were performed at 25 °C under a LD 14: 10 photoperiod centred at 13.00 local time. Animals were acclimated to these conditions for at least 1 week prior to experimentation.

Larvae were swum in an aquatic flow chamber (Vogel & LaBarbera, 1978) in which current velocity could be regulated by varying the propeller speed (see Fig. 1). We calibrated the propeller speed and the flow velocity by timing the passage of dye through the chamber. Screens confined swimming larvae to a small central chamber (25 × 6.5 cm diameter; water depth 5.5 cm) in which the flow was grossly laminar as determined by the dry tracers. The velocity profile of our flow chamber was approximately parabolic. We considered the average velocity (0.5 × the maximum velocity) as the velocity of the water and noted when tadpoles shifted their position in relation to the walls, since a tadpole close to the wall experienced a lower current

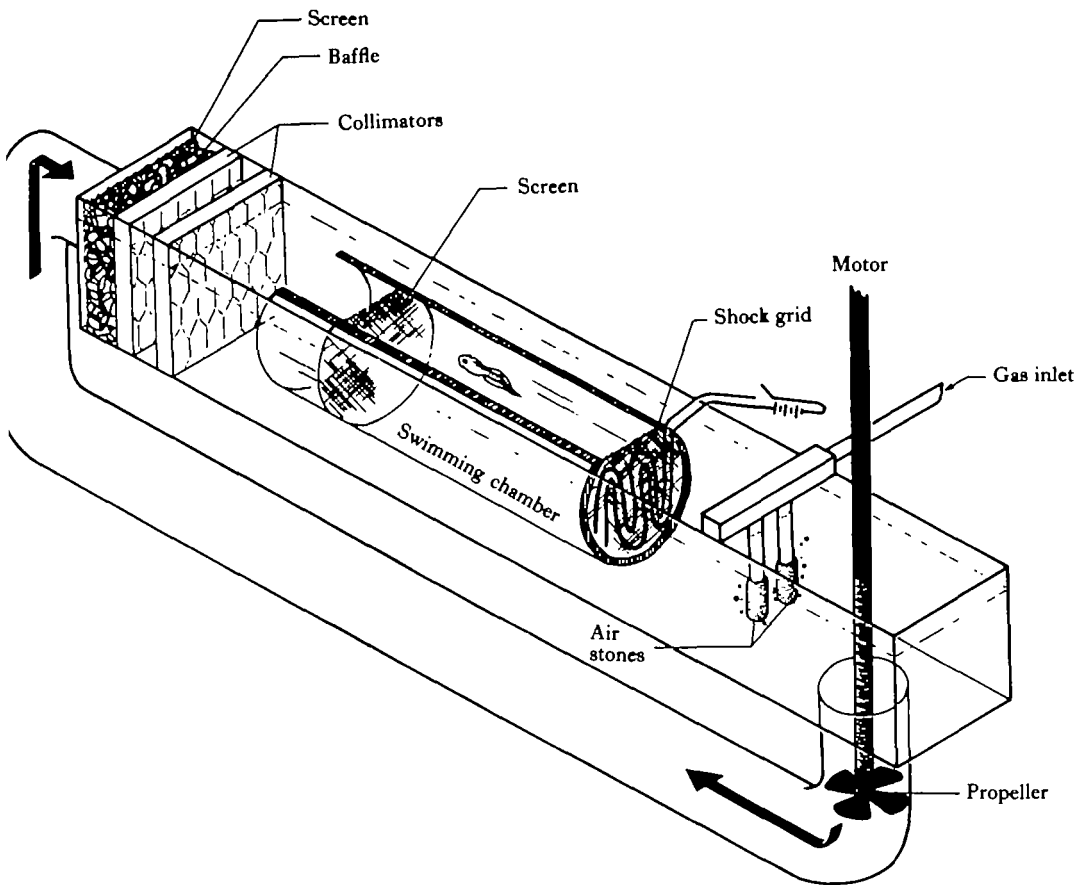


Fig. 1. Flow tank used to measure stamina of anuran larvae. Arrows indicate direction of water flow. Dimensions of the chamber are provided in the text.

velocity. The frontal area of all larvae was less than 1/10 the cross-sectional area of the central chamber; thus no correction was required for solid blocking (see Brett, 1964; Webb, 1975). A wire grid woven into the rear screen of the central chamber was connected to an electric stimulus generator (C. S. Stoelting Co., Chicago, IL). We controlled the partial pressure of oxygen in the water (P_{wO_2}) by gassing the water with air or nitrogen. We measured P_{wO_2} with a YSI O_2 electrode (Yellow Springs Instruments Co., Antioch, OH). Gas outlets (air stones) and the O_2 electrode were positioned downstream from the central chamber.

Performance of larvae was assessed as stamina: i.e., the time (measured with a stopwatch) to exhaustion for a larva swimming at a single speed. Experimental larvae were placed individually in the flow chamber, and flow was initiated 60 s after introduction. Preliminary observations indicated that larvae would often cease swimming and come to rest against the downstream screen before they were completely exhausted; i.e., they could resume swimming for a considerable period immediately after minor stimulation. Hence we used intermittent electrical stimulation to ensure sustained swimming and complete exhaustion. When a larva came to rest against the screen and would not resume swimming upon mild stimulation, we quickly stopped the water flow, gently lifted the larva off the screen, and directed its head upstream. Flow was immediately re-initiated and intervals with the current off (usually about 5 s) were subtracted from total stamina. Larvae were considered exhausted the third time they were unable to sustain swimming for 6 s or more after having to be returned manually to the current.

We took care to use the minimum electrical stimulus to which larvae responded. Inasmuch as stimulus intensity varied with position of the larvae and corrosion of the shock grid, we made no effort to quantify the stimulus intensity received by the larvae.

We measured aerial respiratory frequency (fL) for *Rana* and *Xenopus* by counting instances in which swimming larvae surfaced to breathe air during a timed interval. We measured branchial irrigation frequency (fG) for *Xenopus* larvae by counting buccal pump strokes in timed intervals.

For each species we measured stamina (and respiratory frequency where appropriate) at constant flow velocities throughout a range of P_{wO_2} . All larvae within most experiments were of similar size, to minimize size-related differences in stamina and ventilatory frequency. Specific velocities for these experiments were selected by initial trial and error so that tadpoles of various size and species became exhausted within minutes, rather than either immediately or within hours. In most cases larvae were free to breathe air during the experiment. In some experiments, however, we placed a barrier on the water's surface, which prevented aerial respiration.

For *Bufo* larvae, stamina was much more variable at high P_{wO_2} than at low P_{wO_2} (Cochran's Q ; $P < 0.05$). The same was true of fL in *Xenopus* larvae. To equalize variance in subsequent analyses, *Bufo* stamina and *Xenopus* fL values were transformed to their logarithms (base 10). No such transformation was necessary in other cases.

We used stepwise multiple regression to analyse effects of P_{wO_2} , body size and ventilatory frequency on stamina. These variables were entered in the first step, dummy variables representing all two-way interactions were entered in the subsequent steps, and a dummy variable representing three-way interaction was

entered in the final step. We are aware of the difficulties of reconciling multivariate analysis with biological patterns of interest to the experimental biologist, and therefore we discuss the results in simplified form with minimal reference to the statistical analyses themselves. Statistics, including the multiple regression equations, are presented in tabular form and provide a more precise statement of the results.

RESULTS AND DISCUSSION

Effect of size on stamina and aerial respiration

In *Rana* and *Xenopus*, the body sizes of larvae were positively correlated with stamina (Table 1). By contrast, within the two groups of *Bufo* larvae in Table 1, both large and small individuals had similar stamina. This apparent discrepancy among the species may be due more to similarity in the sizes of the *Bufo* larvae chosen for the experiments than to any real interspecific difference.

Body size had little or no effect on the fL of both *Rana* and *Xenopus* larvae. For *Rana*, r was 0.37 ($N = 41$, $P < 0.05$). For *Xenopus*, r was 0.10 ($N = 18$, $P > 0.05$).

Effect of Pw_{O_2} and aerial respiration on stamina

The Pw_{O_2} , fL and body size (see above) markedly affected the stamina of 45 *Rana* larvae (5.6–26.2 mg dry mass). All of these variables were related to one another in a complex way as seen in Fig. 2. As the Pw_{O_2} declined, the stamina of the larvae decreased. This decrease, however, was more marked in large larvae than in small larvae. These effects were apparent at all fL values. Increases in fL increased the stamina of larvae, proportionally more at low Pw_{O_2} than at high Pw_{O_2} but absolutely more so at high Pw_{O_2} than at low Pw_{O_2} . The absolute shift was, again, more pronounced in large larvae than in small larvae. Thus, Pw_{O_2} , body size and fL each individually and collectively are positively correlated with stamina. The collective effects, however, are greater than the individual effects, as indicated by the highly significant interaction terms in the multiple regression equation (Table 2).

Given that fL was positively correlated with stamina for *Rana* larvae one would predict that experimental modification of fL would alter larval stamina, especially for

Table 1. *Bivariate correlation between tadpole size and stamina*

Species	N	Dry mass (mg) $\bar{x} \pm s.d.$	Correlation coefficient r	Probability P
<i>Rana berlandieri</i> (swum at various Pw_{O_2})	45	12.8 \pm 1.2	0.48	0.01
<i>Bufo americanus</i> (small)	103	4.2 \pm 0.1	0.03	n.s.
<i>Bufo americanus</i> (large)	27	7.6 \pm 0.3	0.16	n.s.
<i>Xenopus laevis</i> (with access to air)	145	35.3 \pm 1.5	0.64	0.01
<i>Xenopus laevis</i> (without access to air)	11	30.6 \pm 3.5	0.60	0.05

n.s. = not significant.

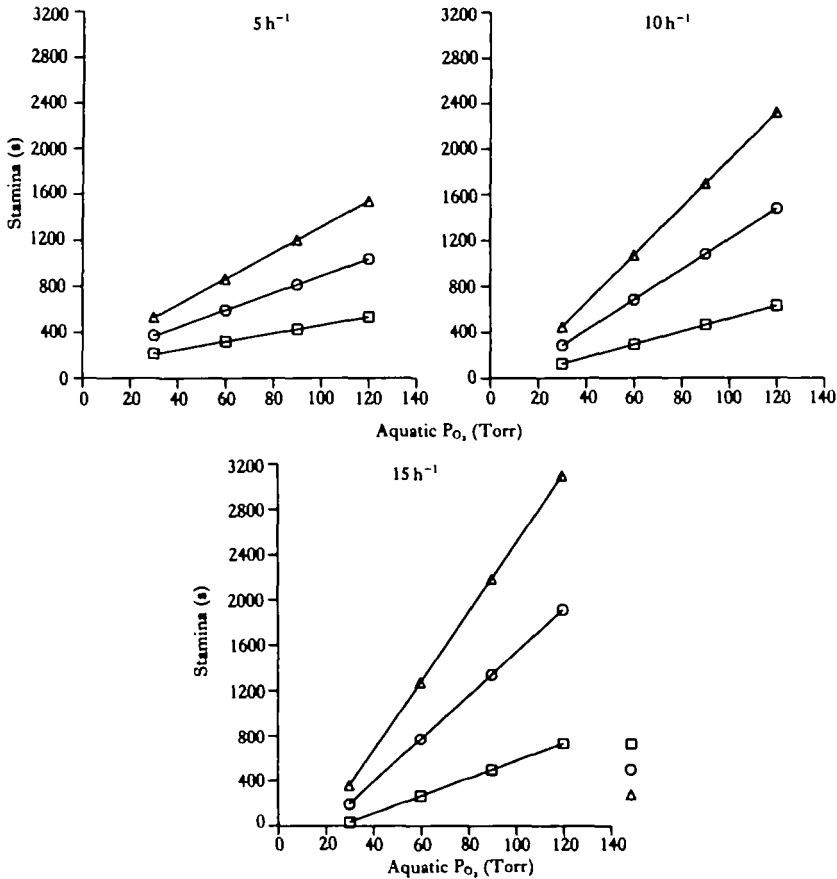


Fig. 2. Effect of aquatic P_{O_2} , body size and aerial respiratory frequency on locomotor stamina of *Rana berlandieri* larvae. Stamina for 45 larvae was analysed with multiple regression (Table 2). The graphs were then generated by substituting typical values for these larvae into the multiple regression equation. The graphs are for larvae of three different sizes ($\square = 12$ mg dry mass, $\circ = 18$ mg dry mass, $\triangle = 24$ mg dry mass) and at three different aerial respiratory frequencies (5, 10 and 15 h^{-1}). Swimming speed was 11.6 cm s^{-1} .

tadpoles swimming at low $P_{W_{O_2}}$. We undertook three experiments to test this hypothesis. In the first test, we observed the fL in three groups of *Rana* larvae swimming at a variety of $P_{W_{O_2}}$. The first group ($N = 41$, dry wt 21.6 ± 1.4 mg) swam for at least 900 s, at which point the observations were terminated. These larvae increased their fL significantly above levels reported for larvae in still water (Feder, 1983), and this increase was greatest at low $P_{W_{O_2}}$ (Fig. 3A, Table 3). The second group of *Rana* larvae were of similar size ($N = 13$, dry wt 20.7 ± 3.3 mg) but fatigued in less than 900 s (Fig. 3B). Although these larvae also increased their fL in response to aquatic hypoxia, the fL of these fast-fatiguing larvae was significantly less than the fL of the larvae in the first group ($P < 0.01$, analysis of covariance). Evidently individual variation in the tendency of larvae to breathe air resulted in differences in stamina.

The third group of larvae were larger ($N = 11$, dry wt 59.4 ± 3.3 mg) and all were able to sustain swimming for 900 s. As with the smaller larvae that did not fatigue, the fL of these larger larvae was greater than the fL for larvae of similar size in still water.

Table 2. Effect of Pw_{O_2} , dry mass and aerial respiration rates (fL) on stamina in anuran larvae

Species Variables	Change in R^2 *	Simple correlation coefficient r
<i>Rana berlandieri</i>		
Main effects		
Mass	23 %	0.48
Pw_{O_2}	8 %	0.17
fL	1 %	0.32
Two-way interaction		
$Pw_{O_2} \times$ fL	15 %	0.48
Mass \times fL	13 %	0.36
Mass \times Pw_{O_2}	5 %	0.53
Three-way interaction		
Mass \times $Pw_{O_2} \times$ fL	13 %	0.60
Total explained variance	78 %	
Stamina = $25.5(\text{Mass}) + 1.4(Pw_{O_2}) + 14(\text{fL}) - 1.1(Pw_{O_2})(\text{fL}) - 3.7(\text{Mass})(\text{fL}) + 0.01(\text{Mass})(Pw_{O_2}) + 0.13(\text{Mass})(Pw_{O_2} \times \text{fL}) - 47.4$		
Small <i>Bufo americanus</i> (regression with \log_{10} stamina)		
Main effect		
Pw_{O_2}	38 %	0.61
Mass	3 %	0.03
Two-way interaction		
$Pw_{O_2} \times$ Mass	1 %	0.61
Total explained variance	41 %	
\log_{10} Stamina = $6.47 \times 10^{-2}(Pw_{O_2}) - 1.02 \times 10^{-2}(\text{Mass}) - 4.89 \times 10^{-5}(Pw_{O_2})(\text{Mass}) + 1.3389$		
Large <i>Bufo americanus</i> (regression with \log_{10} stamina)		
Main effects		
Pw_{O_2}	49 %	0.70
Mass	1 %	0.16
Two-way interaction		
$Pw_{O_2} \times$ Mass	n.s.	-
Total explained variance	50 %	
\log_{10} Stamina = $4.63 \times 10^{-2}(Pw_{O_2}) + 1.59 \times 10^{-2}(\text{Mass}) + 1.4837$		
<i>Rana berlandieri</i> (swum at 11.6 cm s^{-1} without access to air)		
Main effects		
Pw_{O_2}	76 %	0.87
Mass	4 %	0.35
Two-way interaction		
$Pw_{O_2} \times$ Mass	1 %	0.90
Total explained variance	81 %	
Stamina = $1.95 \times 10^{-3}(Pw_{O_2}) - 4.87 \times 10^{-3}(\text{Mass}) + 2.59 \times 10^{-4}(Pw_{O_2})(\text{Mass}) + 2.36$		
* Change in R^2 = (increase in the multiple coefficient of determination, R^2 , due to addition of the indicated variable to the multiple regression equation) \times 100 %. In other words, 'change in R^2 ' is the increase in percentage of explained variation due to incorporation of a variable into the regression equation.		
n.s. = not significant.		
Pw_{O_2} is in Torr, dry mass is in mg; fL in h^{-1} ; stamina in s. Independent variables and interaction terms were entered into stepwise multiple regression in the order shown.		

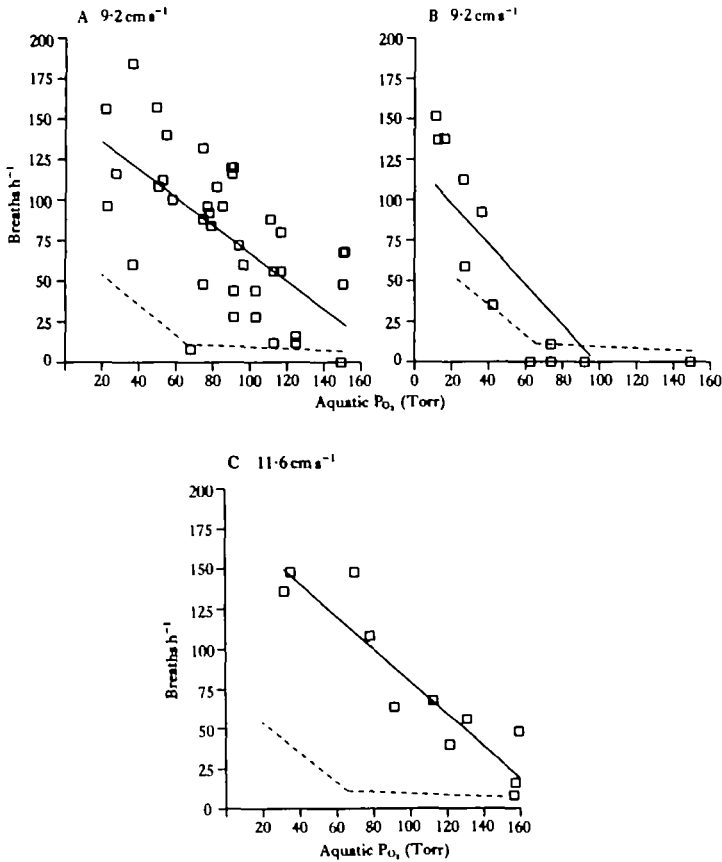


Fig. 3. Effect of aquatic P_{O_2} on aerial respiratory frequency (fL) of *Rana berlandieri* larvae. In each graph, the solid line is a linear regression of the data and the broken lines are the fL values for larvae in still water (Feder, 1983). (A) Larvae that swam for 900 s without exhaustion ($N = 41$). (B) Larvae that fatigued in less than 900 s ($N = 13$). (C) Larger larvae than in (A) and (B), all of which swam for 900 s ($N = 11$). Current velocities are presented above the graphs. Regression equations for swimming larvae are given in Table 3.

and again the larger larvae increased fL in response to declining $P_{W_{O_2}}$ (Fig. 3C).

Our second test of the hypothesis that aerial respiration increases larval stamina used *Bufo* instead of *Rana*. *Bufo* larvae lack functional lungs and do not breathe air (Wassersug & Seibert, 1975); thus aquatic hypoxia should decrease their swimming ability even more than in *Rana* larvae. Our experiment here used both 'small' ($N = 103$, dry wt 4.2 ± 0.1 mg) and 'large' ($N = 27$, dry wt 7.6 ± 0.3 mg) *Bufo* larvae at comparable relative speeds at a variety of $P_{W_{O_2}}$. As expected, the stamina of both groups of *Bufo* declined markedly at low $P_{W_{O_2}}$ (Fig. 4, Table 2). *Bufo* larvae show a much simpler relationship between $P_{W_{O_2}}$ and stamina than the rather complex interaction of these factors in *Rana*. For *Bufo*, stamina conformed to the $P_{W_{O_2}}$.

Our final test involved preventing swimming *Rana* tadpoles from using their lungs. We reasoned that this would cause their stamina to conform to the $P_{W_{O_2}}$, as in *Bufo*. *Rana* larvae were swum at various $P_{W_{O_2}}$ in the flow tank while a barrier on the water's

Table 3. Effect of Pw_{O_2} and dry mass on aerial respiration rates (fL) of swimming *Rana* and *Xenopus* larvae

Species Variables	Change in R^2	Simple correlation coefficient r
<i>Rana berlandieri</i>		
Main effects		
Pw_{O_2}	46 %	-0.67
Mass	4 %	0.37
Two-way interaction		
$Pw_{O_2} \times \text{Mass}$	1 %	-0.17
Total explained variance	50 %	
Respiratory rate (fL) = $-0.86(Pw_{O_2}) + 153.1$		
<i>Xenopus laevis</i> (\log_{10} fL)		
Main effects		
Pw_{O_2}	71 %	-0.84
Mass	1 %	0.10
Two-way interaction		
$Pw_{O_2} \times \text{Mass}$	1 %	0.78
Total explained variance	71 %	

$$\log_{10} \text{ respiratory rate (fL)} = -6.25 \times 10^{-3}(Pw_{O_2}) + 1.2581$$

See Table 2 for explanation of symbols. *Rana*, $N = 41$; *Xenopus*, $N = 18$.

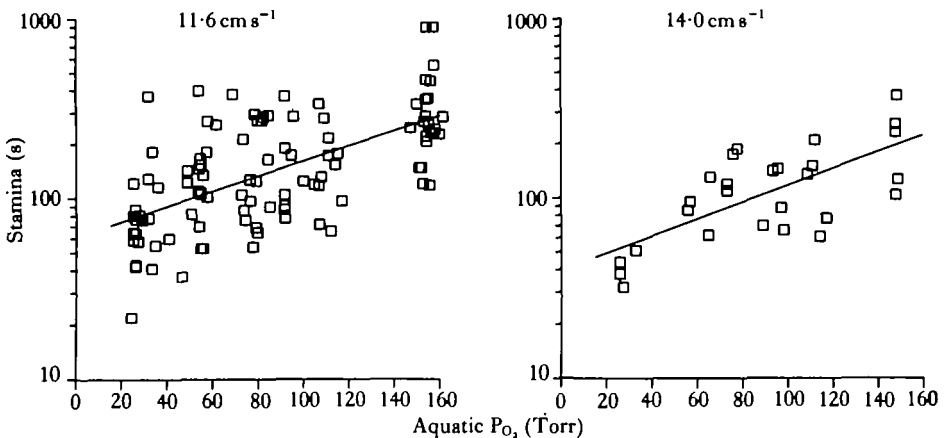


Fig. 4. Effect of aquatic P_{O_2} on locomotor stamina of 'small' (left) and 'large' (right) *Bufo* larvae swum at similar relative velocities. Velocities are presented above the graphs. Regression equations for plotted lines are given in Table 2.

surface eliminated the tadpoles' access to air. In these functionally lungless *Rana* larvae stamina declined as the experimental Pw_{O_2} declined. The complex interaction between body size, Pw_{O_2} , and stamina seen before, when the larvae could breathe air, was no longer evident. Even in normoxic water, curtailing aerial respiration decreased stamina for *Rana* larvae. This was demonstrated by six larvae that were denied access to air for 4 h prior to and during measurement of their stamina. Compared to a control

group of 12 larvae that had continual access to air but were otherwise similar in size [$F_{(1,13)} = 0.14$; $P = 0.932$], the functionally lungless larvae had significantly lower stamina [$F_{(1,13)} = 5.5$; $P = 0.035$].

The foregoing results for *Rana* and *Bufo* suggest that (1) P_{wO_2} affects swimming performance of anuran larvae; (2) larvae with functional lungs can (and do) buffer these effects to some extent through aerial respiration; and (3) effects of P_{wO_2} and fL are most pronounced in large larvae.

Stamina and aerial respiration in Xenopus, a buoyant tadpole

Active *Rana* and *Xenopus* were similar in several of their respiratory responses to aquatic hypoxia. *Xenopus* larvae ($N = 18$, dry wt 22.2 ± 1.2 mg) increased their fL above resting levels recorded for *Xenopus* larvae in still water (Feder & Wassersug, 1983). In addition, swimming *Xenopus* larvae increased their fL in response to aquatic hypoxia (Fig. 5, Table 3). Larvae in still water also increased their fL in response to a low P_{wO_2} (Feder & Wassersug, 1983).

Attempts to demonstrate the additional response patterns shown above for *Rana* and *Bufo* on larvae of *Xenopus* met with mixed success. Here we present data to suggest that the buoyancy afforded by the lungs in *Xenopus* larvae and the drag due to branchial irrigation (see following section) offset the potential increases in stamina that might result from increased oxygen consumption.

In larval *Xenopus*, lung ventilation and the resultant buoyancy interfered with sustained swimming. As *Xenopus* larvae swimming in our flow chamber surfaced to breathe air, their flattened bodies were perpendicular to the current. This posture resulted in the animals being swept along by the current. The animals that surfaced frequently were swept into the screen at the end of the flow chamber. Even the animals

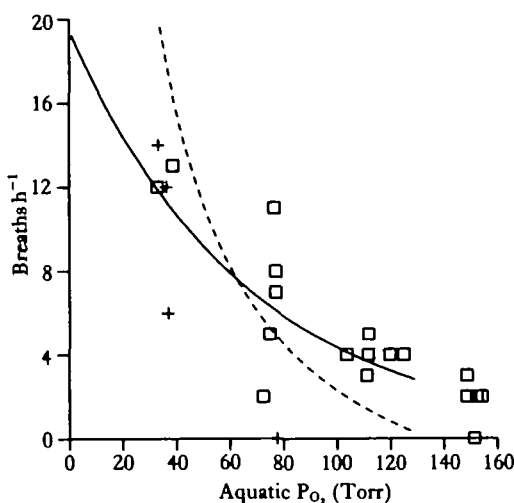


Fig. 5. Effect of aquatic P_{O_2} on aerial respiratory frequency (fL) of *Xenopus* larvae. The solid curve is a fit of \log fL against P_{O_2} for all swimming animals ($N = 21$); the broken line is a similar fit for larvae ($N = 122$) in still water (Feder & Wassersug, 1983). Symbols indicate whether individual larvae did (\square , $N = 18$) or did not ($+$, $N = 3$) sustain swimming for 900 s. Current velocity was 9.2 cm s^{-1} . Regression equation for swimming animals is given in Table 3.

that maintained their position pitched and yawed considerably. When the *Xenopus* larvae breathed air, they released a gas bubble after inspiration. Larvae swimming in a strong current were often unable to release this bubble; such larvae were exceedingly buoyant and unable to maintain either their trim or their position in the current. As soon as these larvae released the air bubble they resumed normal swimming. Additionally, the fL of these active animals was lower than the fL of animals of comparable size in still water at low P_{wO_2} (Fig. 5). No distinction was evident between the fL of the larvae that became fatigued and the fL of the larvae that were able to swim for 900 s (Fig. 5).

These initial observations on the effects of buoyancy suggested that aerial respiration actually hinders swimming ability in *Xenopus* larvae. To test this hypothesis, we prevented 11 *Xenopus* larvae from using their lungs by excluding them from the air in the flow chamber for 2 h before and during measurement of their stamina. Although their water was aerated continuously, these larvae became negatively buoyant during this period of forced submergence. The stamina of these larvae was significantly greater than that of 145 similar larvae allowed access to air [analysis of covariance ($F_{(1,154)} = 30.4$; $P < 0.01$), Fig. 6]. These larvae, which were functionally lungless and evidently negatively buoyant, swam unusually close to the bottom, where current velocity was lowest.

Although aerial respiration was detrimental to the stamina of larval *Xenopus* at a high P_{wO_2} , inflated lungs encumbered swimming less at a low P_{wO_2} . An experiment involving 11 *Xenopus* verified this. Each larva was swum in the flow chamber on two consecutive days. On one day they swam in normoxic water and had access to air. On the other day, each swam in hypoxic water ($P_{\text{wO}_2} = 60$ Torr) and had no access to air.

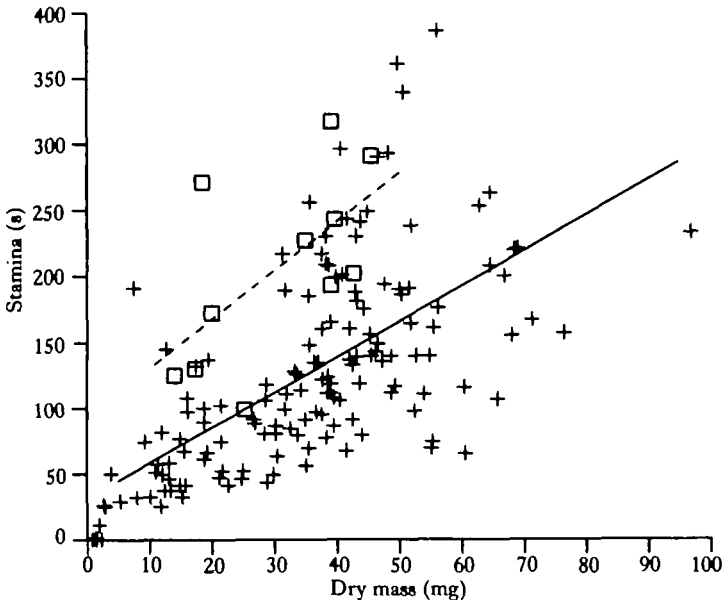


Fig. 6. Effect of aquatic P_{O_2} on locomotor stamina of *Xenopus* larvae with and without access to air. Solid line is for animals that had access to air; broken line is for animals without access to air. Current velocity was 11.6 cm s^{-1} . + = lungs inflated, □ = no access to air.

The order of presentation of these two treatments was reversed in half of the animals. The larvae had much lower stamina on the day in which they swam in hypoxic water without access to air than on the day in which they swam in normoxic water with the possibility of aerial respiration ($P = 0.012$, Sign Test); the average reduction in stamina was 51%. This experiment was repeated with 15 larvae, but animals in hypoxic water were allowed access to air. In this case, the average reduction in stamina of the hypoxic animals was only 25% ($P = 0.036$, Sign Test). For *Xenopus* swimming in hypoxic water, the decrease in stamina seen in animals allowed access to air was less than in animals with no possibility of aerial respiration, but the difference was not significant (Mann-Whitney U ; $P = 0.096$).

Stamina and aquatic respiration in Xenopus

In *Xenopus*, branchial respiration, like aerial respiration, may have consequences that lead to reduced stamina. The orientation of the mouth, which is large and anteriorly directed in *Xenopus* larvae, may affect larval stamina through its effects on pressure drag. When the mouth is open the frontal area of a *Xenopus* larva increases greatly. In *Rana*, *Bufo*, and, indeed, most anuran larvae, the mouth is relatively small

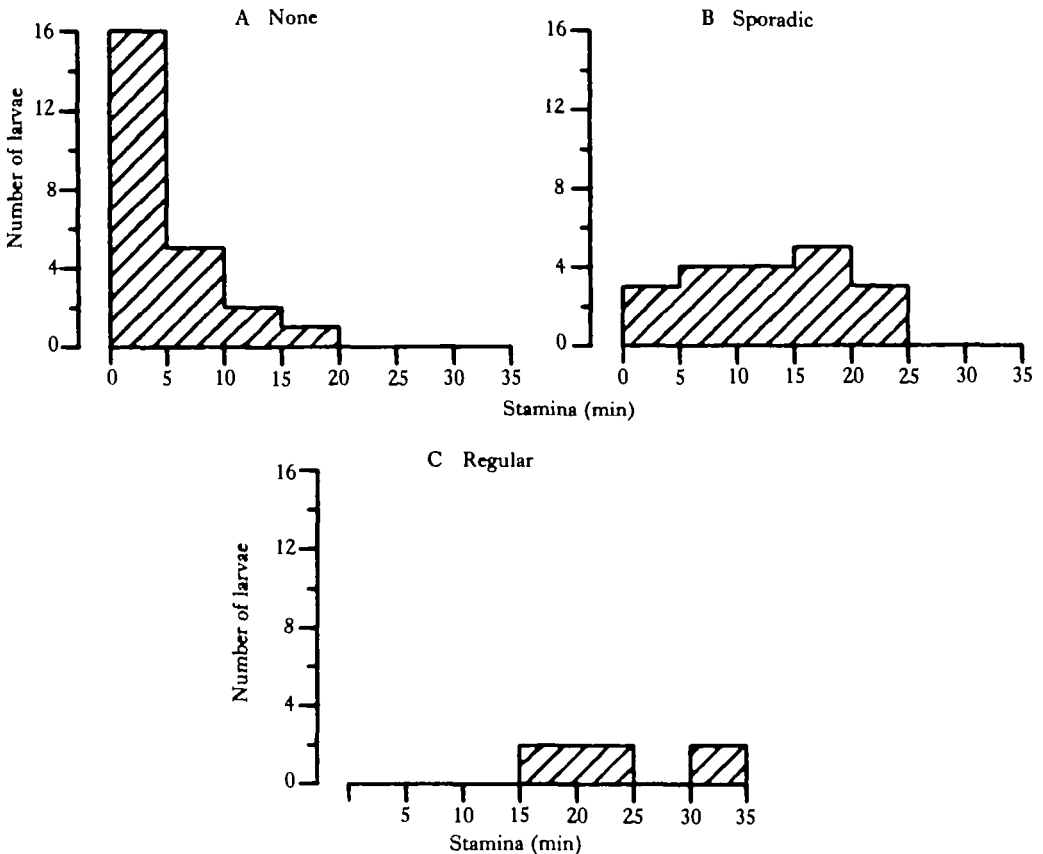


Fig. 7. Histograms of number of *Xenopus* larvae showing no, sporadic and regular branchial irrigation in relation to locomotor stamina. Current velocity was 11.6 cm s^{-1} .

and directed ventrally. Opening the mouth changes the frontal area of these larvae negligibly. Branchial irrigation must necessarily cause a much greater increase in pressure drag on *Xenopus* than on *Rana* or *Bufo* larvae.

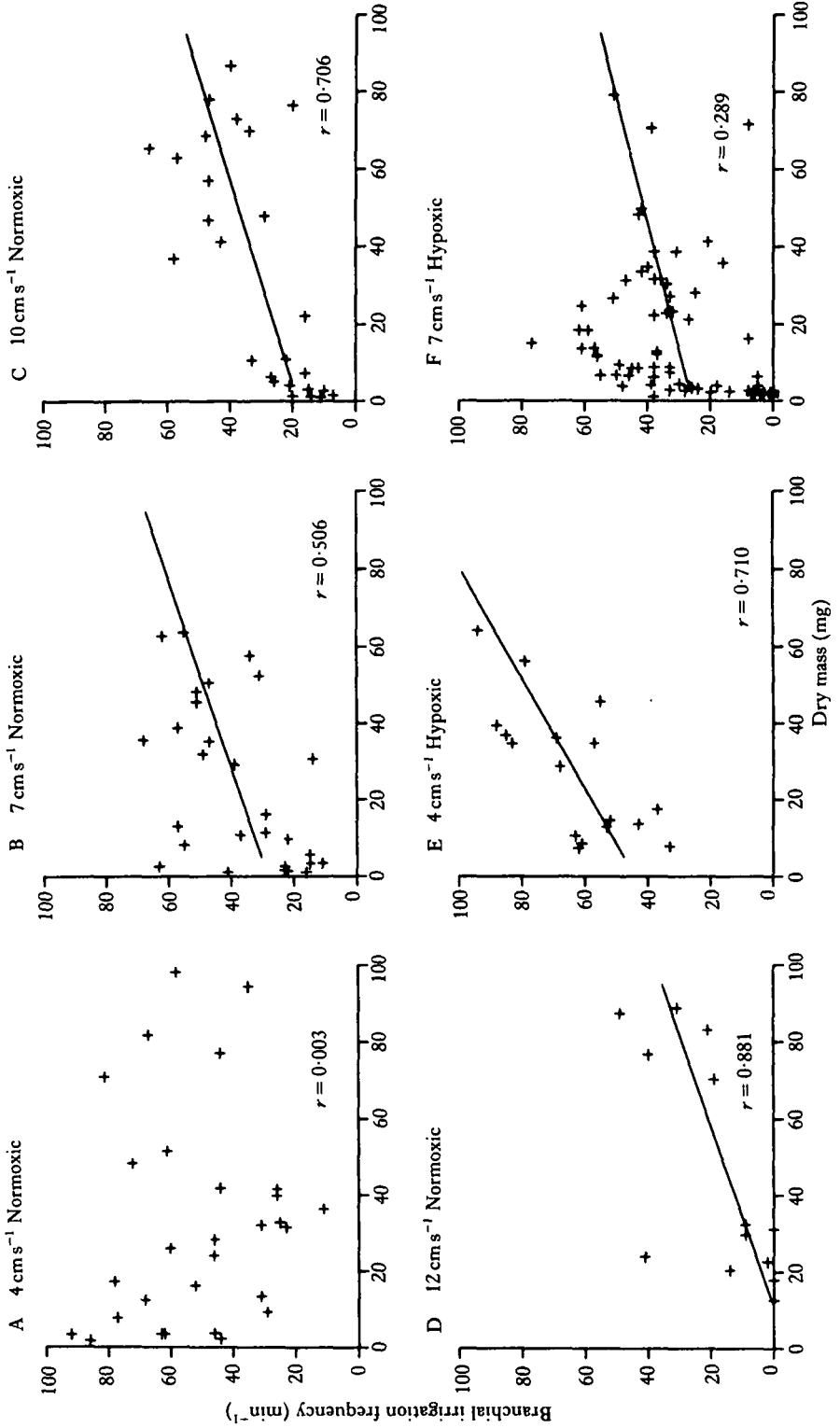
Although branchial irrigation may affect drag in *Xenopus* larvae, the tendency to irrigate their gills was positively related to sustained swimming in these larvae. Our initial observations revealed that *Xenopus* tadpoles swam in two relatively distinct modes. At one extreme, there was little or no branchial irrigation, lateral displacement occurred along the whole length of the tail and the trunk, and the animals fatigued rapidly (Fig. 7A, B). In the other, branchial irrigation was regular and continuous, lateral undulation was limited to the distal portion of the tail, and stamina was high (Fig. 7C). The choice of mode was apparently voluntary; animals switched between modes during a single bout of swimming. Nonetheless, those animals that failed to irrigate their buccopharyngeal surfaces fatigued rapidly and those with regular branchial irrigation had high stamina.

We observed characteristic reductions in the fG of *Xenopus* larvae that were associated with both current velocity and tadpole body size; these reductions may have been associated with declines in stamina. In order to assess more rigorously the relationship between fG, flow velocity and tadpole body size, we measured branchial irrigation frequency for *Xenopus* larvae of different sizes in aerated water flowing at 4, 7, 10 and 12 cm s⁻¹ (sample size ranged from 13 to 29, see Fig. 8A–D). In still water, the fG of the *Xenopus* larvae appeared to vary randomly with the PwO₂ and body size, and may have been more directly related to the concentration of particulate matter in the water (Seale, Hoff & Wassersug, 1981; Feder & Wassersug, 1983). Similar random variation was evident for the larvae at the lowest current velocity that we considered, 4 cm s⁻¹ (Fig. 8A). At this velocity, the fG was unrelated to the body size of the larvae. At higher current velocities, body size has a distinct effect upon the fG of the larvae (Fig. 8B–D). The intensity of this size effect was similar for the larvae swum at 7, 10 and 12 cm s⁻¹ [analysis of covariance, $F_{(1,61)} = 0.1$; $P = 0.8$].

Swimming at a high speed reduced the fG of the *Xenopus* larvae [$F_{(2,61)} = 23.0$; $P < 10^{-5}$]. This effect is evident in comparing the height of the lines in Fig. 8B–D. Inasmuch as small body size also reduces the fG of *Xenopus* larvae (see above), the small larvae in particular tended to have a low fG under many flow regimes and this was associated with reduced stamina (Fig. 7).

Additional measurements of fG were made for *Xenopus* larvae swimming at 4 ($N = 17$) and 7 ($N = 71$) cm s⁻¹ in hypoxic water (30–35 Torr PwO₂). Decreasing PwO₂ was similar to increasing current velocity in its effects on fG. In larvae swum at 4 cm s⁻¹, body size had a negligible effect upon stamina in normoxic water, but body size affected the stamina of *Xenopus* in hypoxic water strongly and positively (Fig. 8E). Larvae swum at the higher velocity in hypoxic water (Fig. 8F) showed a lower fG than the larvae swum in normoxic water (Fig. 8B). Thus, aquatic hypoxia may have affected stamina indirectly (by altering the fG and hence the hydrodynamic properties of swimming larvae), as well as directly by altering oxygen uptake.

We have no indication whether decreased fG is a cause or a symptom of locomotor fatigue in *Xenopus*. Decreases in fG may both reduce drag and reduce O₂ uptake. Nonetheless, small size, aquatic hypoxia and high swimming velocity are clearly associated with reduced stamina and reduced fG in *Xenopus* tadpoles.



DISCUSSION

Our data demonstrate that respiratory patterns may have major effects, both positive and negative, on locomotor capacities, and that locomotion may likewise alter respiratory patterns.

The increased stamina for larvae with access to air demonstrates the positive contribution of aerial respiration to sustained activity in some cases. This result is not surprising, for any increase in the O_2 uptake of an organism may affect its locomotor ability (Davies, Foster, Warren & Doudoroff, 1963; Dahlberg, Shumway & Doudoroff, 1968; Kutty, 1968; Jones, 1971; Smit, Amelink-Koutstaal, Vijverberg & von Vaupel-Klein, 1971). We know of no previous study in which aerial respiratory rates and stamina were measured concurrently, although air-breathing is a common response to aquatic hypoxia (Johansen, 1970; Hughes, 1976; Randall, Burggren, Farrell & Haswell, 1981). Tadpoles increase fL in response to activity in much the same way that larval amphibians (Wassersug & Seibert, 1975; Branch & Taylor, 1977; R. J. Wassersug & M. E. Feder, 1979, unpublished data; West & Burggren, 1982) and facultatively air-breathing fish (Holeton, 1980) augment fL in response to aquatic hypoxia. Under a combination of hypoxia and activity this increase in fL is most dramatic.

The absence of air-breathing in certain larvae, such as *Bufo*, and increased stamina in other larvae, such as *Xenopus*, when denied access to air suggests that specific costs are also associated with aerial respiration. One of these costs is related to buoyancy. Carrying air under water increases an organism's buoyancy and may decrease its stability in a current. These effects are not negligible. Gee (1977) has shown that buoyancy is related to current velocity in fish, and that many fish actively modify internal gas volumes in response to different flow regimes. One inference of Gee's work is that homeostasis of buoyancy has priority above respiratory responses to activity and hypoxia in many fish; i.e., respiratory parameters are regulated to maintain a constant buoyancy, even though O_2 uptake may be affected. In an air-breathing larva, this priority is evidently reversed, as active *Rana* and *Xenopus* larvae undergo changes in buoyancy; larvae increase both the amplitude and frequency of tail undulation in response to experimental changes in buoyancy (Van Bergeijk, 1959). A similar reversal in priorities, including increases in both variation in buoyancy and fin movements, is seen in mudminnows (*Umbra limi*) in hypoxic water (Gee, 1981).

Branchial O_2 exchange also has both benefits and costs; however, our data suggest that in anuran larvae the latter are more significant. Anuran larvae have limited abilities to extract O_2 from water (West & Burggren, 1982; Feder, 1983; Feder & Wassersug, 1983). At low P_{wO_2} , larvae in fact lose O_2 to the water, an occurrence that is rarely reported for other aquatic vertebrates. Not only is aquatic O_2 uptake ineffective, it may be energetically expensive as well. No measurements of respiratory costs are available for anuran larvae; in fish, however, the cost of branchial irrigation

Fig. 8. Effect of body size, current velocity and aquatic P_{O_2} on branchial irrigation frequency in *Xenopus* larvae. The correlation coefficient (r) and a linear regression of the data (where appropriate) are provided for each graph. Experimental flow rate and oxygen concentration are indicated above each figure.

averages 8–10% of total metabolism and is often much greater (Holeton, 1980; Freadman, 1981). The buccal pump musculature is massive in most anuran larvae and their gill filters are dense (Wassersug, 1980). Hence the cost of branchial irrigation in anuran larvae is likely to be even greater than in fish. Additionally, in larvae with anteriorly directed mouths (such as *Xenopus*), branchial irrigation also increases drag.

Many of the differences in respiration between amphibian larvae and fish may relate to fundamental differences in their way of life and body form. A typical tadpole has a less streamlined, more globose body than most fish. Anuran larvae lack the pelvic and pectoral fins so important in the regulation of swimming in fish (Gray, 1968; Magnuson, 1978). The tadpole body form is not a morphology associated with sustained activity at constant velocities. The medially deep tail fin of anuran larvae suggests that in general these larvae are better designed for acceleration (burst activity) than sustained swimming [cf. Webb's discussion (1975, 1977, 1981) of fish shape and swimming mode]. In contrast to fish, no tadpoles cruise continually in open water in search of prey. Indeed, in our flow chamber, larvae change velocity often, dashing forward and then drifting back, rather than cruising at a constant speed.

Anuran larvae, in lacking the scales characteristic of fish, may have superior abilities to exchange gas through their skin. Contrary to a recent suggestion by de Saint-Aubain (1982) that tadpoles have a reduced ability to utilize gaseous exchange through the skin, as much as 70% of the oxygen uptake by anuran larvae can be cutaneous (Burggren & West, 1982; Burggren, Feder & Pinder, 1983). Moreover, because cutaneous respiration does not involve specialized ventilatory structures and modes of behaviour in most amphibians, its energetic cost may be low (Burggren *et al.* 1983).

One disadvantage of cutaneous respiration is the relative vulnerability of the respiratory surface to injury. Another is that, unless an organism changes shape with increasing size, a cutaneous respiratory surface may become increasingly ineffective as an organism grows (Ultsch, 1976). In our experiments, air breathing increased the stamina for large larvae more than for small ones. This finding is consistent with the hypothesis that O₂ exchange at the skin limits activity in anuran larvae. The small body sizes of functionally lungless larvae, such as *Bufo*, (Wassersug & Seibert, 1975) also consistent with limitation of gas exchange by cutaneous surface area.

The morphology of anuran larvae actually complicates the ventilation of lungs and gills during swimming. Most anuran larvae are demersal, have large branchial baskets, and have a ventrally directed mouth. All these features, in both fish (Roberts, 1975) and anuran larvae, preclude ram irrigation. The position of the mouth in most larval anurans and the absence of lateral locomotor appendages must make breathing air a more awkward activity for an anuran larva than a fish. Because of slow transit times and awkwardness, larval anurans are probably more susceptible to predation at the water's surface than are most fish. Anurans at metamorphosis are particularly inefficient swimmers (Wassersug & Sperry, 1977) and are especially susceptible to predation (Arnold & Wassersug, 1978). The commitment to metamorphosis for the anuran larva evidently required compromises in form and function that did not figure in the evolution of most teleostean fish.

There have been relatively few experimental studies of the links between functional

specializations and performance (e.g., Webb, 1977; Bennett, 1980; Kimmel, Eaton & Powell, 1980; Silberglied, Aiello & Windsor, 1980). As our differing results for *Rana* and *Xenopus* larvae demonstrate, similar roles of pulmonary respiration in allowing high rates of oxygen consumption (Feder, 1983; Feder & Wassersug, 1983) do not necessarily result in similar measures of performance. We hope that our work will stimulate additional investigations in this vein.

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REFERENCES

- ARNOLD, S. J. & WASSERSUG, R. J. (1978). Differential predation by garter snakes (*Thamnophis*): Social behavior as a possible defense. *Ecology* **59**, 1014–1022.
- ARUNACHALAM, S., VIVEKANANDAN, E. & PANDIAN, T. J. (1976). Food intake, conversion and swimming activity in the air-breathing catfish, *Heteropneustes fossilis*. *Hydrobiologia* **51**, 213–217.
- BENNETT, A. F. (1980). The thermal dependence of lizard behaviour. *Anim. Behav.* **28**, 752–762.
- BRANCH, L. C. & TAYLOR, D. H. (1977). Physiological and behavioral responses of larval spotted salamanders (*Ambystoma maculatum*) to various concentrations of oxygen. *Comp. Biochem. Physiol.* **58A**, 269–274.
- BRETT, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Bd Can.* **21**, 1183–1226.
- BURGGREN, W. W., FEDER, M. E. & PINDER, A. W. (1983). Temperature and the balance between aerial and aquatic respiration in larvae of *Rana berlandieri* and *Rana catesbeiana*. *Physiol. Zool.* **55**.
- BURGGREN, W. W. & WEST, N. H. (1982). Changing respiratory importance of gills, lungs and skin during metamorphosis in the bullfrog, *Rana catesbeiana*. *Respir. Physiol.* **47**, 151–164.
- DAHLBERG, M. L., SHUMWAY, D. L. & DOUDOROFF, P. (1968). Influence of dissolved oxygen and carbon dioxide on swimming performance of largemouth bass and coho salmon. *J. Fish. Res. Bd Can.* **25**, 49–70.
- DAVIES, G. E., FOSTER, J., WARREN, C. E. & DOUDOROFF, P. (1963). The influence of oxygen concentration on the swimming performance of juvenile Pacific Salmon at various temperatures. *Trans. Am. Fish. Soc.* **92**, 111–124.
- DEJOURS, P. (1976). Water versus air as the respiratory medium. In *Respiration of Amphibious Vertebrates*, (ed. G. M. Hughes). New York: Academic Press.
- DRUMMOND, H. M. (1980). *Aquatic Foraging in Some New World Natricine Snakes: Generalists and Specialists and their Behavioral Evolution*, Ph.D. dissertation, University of Tennessee, Knoxville.
- FEDER, M. E. (1981). Effects of body size, trophic state, time of day, and experimental stress on oxygen consumption of anuran larvae: an experimental assessment and evaluation of the literature. *Comp. Biochem. Physiol.* **70A**, 497–508.
- FEDER, M. E. (1983). Responses to acute aquatic hypoxia in larvae of the frog *Rana berlandieri*. *J. exp. Biol.* **104**, 79–95.
- FEDER, M. E. & WASSERSUG, R. J. (1983). Aerial versus aquatic oxygen consumption in larvae of the Clawed Frog, *Xenopus laevis* (in press).
- FREADMAN, M. A. (1981). Swimming energetics of striped bass (*Morone saxatilis*) and bluefish (*Pomatomus saltatrix*): hydrodynamic correlates of locomotion and gill ventilation. *J. exp. Biol.* **90**, 253–266.
- GEE, J. H. (1977). Effects of size of fish, water temperature and water velocity on buoyancy by fathead minnows, *Pimephales promelas*. *Comp. Biochem. Physiol.* **56A**, 503–508.
- GEE, J. H. (1981). Coordination of respiratory and hydrostatic functions of the swimbladder in the central mud minnow, *Umbra limi*. *J. exp. Biol.* **92**, 37–52.
- GOSNER, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183–190.
- GRAY, J. (1968). *Animal Locomotion*. World Nat. Ser. London: Weidenfeld and Nicolsen.
- HOLETON, G. F. (1980). Oxygen as an environmental factor of fishes. In *Environmental Physiology of Fishes*, (ed. M. A. Ali). New York: Plenum Press.
- HORA, S. L. (1930). Ecology, bionomics, and evolution of the torrential fauna, with special reference to the organs of attachment. *Phil. Trans. R. Soc. Ser. B* **218**, 241–243.
- HUGHES, G. M. (1976). (ed.). *Respiration of Amphibious Vertebrates*. New York: Academic Press.

- JOHANSEN, K. (1970). Air breathing in fishes. In *Fish Physiology*, Vol. 4, (ed. W. S. Hoar & D. J. Randall). New York: Academic Press.
- JONES, D. R. (1971). The effects of hypoxia and anaemia on the swimming performance of rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **55**, 541-551.
- KIMMEL, C. B., EATON, R. C. & POWELL, S. L. (1980). Decreased fast-start performance of zebrafish larvae lacking Mauthner neurons. *J. comp. Physiol.* **140**, 343-350.
- KRAMER, D. L. & McCLURE, M. (1981). The transit cost of aerial respiration in the catfish *Corydoras aeneus* (Callichthyidae). *Physiol. Zool.* **54**, 189-194.
- KRAMER, D. L. & GRAHAM, J. F. (1976). Synchronous air breathing, a social component of respiration in fishes. *Copeia* **1976**, 689-697.
- KUTTY, M. N. (1968). Influence of ambient oxygen on the swimming performance of goldfish and rainbow trout. *Can. J. Zool.* **46**, 647-653.
- MAGNUSON, J. J. (1978). Locomotion by scombrid fishes: hydromechanics, morphology, and behavior. In *Fish Physiology*, Vol. 7, (ed. W. S. Hoar & D. J. Randall). New York: Academic Press.
- PANDIAN, T. J. & VIVEKANANDAN, E. (1976). Effects of feeding and starvation on growth and swimming activity in an obligatory air-breathing fish. *Hydrobiologia* **49**, 33-39.
- RANDALL, D. J., BURGGREN, W. W., FARRELL, A. P. & HASWELL, M. S. (1981). *The Evolution of Air Breathing in Vertebrates*. Cambridge: Cambridge University Press.
- ROBERTS, J. L. (1975). Active branchial and ram gill ventilation in fishes. *Biol. Bull. mar. biol. Lab., Woods Hole* **148**, 85-105.
- SAINT-AUBAIN, M. L. DE (1982). The morphology of amphibian skin vascularization before and after metamorphosis. *Zoomorphology* **100**, 55-63.
- SEALE, D. B., HOFF, K. & WASSERSUG, R. J. (1981). *Xenopus laevis* larvae (Amphibia, Anura) as model suspension feeders. *Hydrobiologia* **87**, 161-169.
- SILBERGLIED, R. E., AIELLO, A. & WINDSOR, D. M. (1980). Disruptive coloration in butterflies: lack of support in *Anartia fatima*. *Science, N.Y.* **209**, 617-619.
- SMIT, H., AMELINK-KOUTSTAAL, J. M., VIJVERBERG, J. & VON VAUPEL-KLEIN, J. C. (1971). Oxygen consumption and efficiency of swimming goldfish. *Comp. Biochem. Physiol.* **39A**, 1-28.
- ULTSCH, G. R. (1976). Respiratory surface area as a factor controlling the standard rate of O₂ consumption of aquatic salamanders. *Respir. Physiol.* **26**, 357-369.
- VAN BERGEIJE, W. A. (1959). Hydrostatic balancing mechanism of *Xenopus* larvae. *J. acoustical Soc. Am.* **31**, 1340-1347.
- VOGEL, S. & LABARBERA, M. (1978). Simple flow tanks for research and teaching. *Bioscience* **28**, 638-643.
- WASSERSUG, R. J. (1980). Internal oral features of larvae from eight anuran families: Functional, systematic, evolutionary, and ecological considerations. *Univ. Kansas Mus. Nat. Hist. Misc. Publ.* **68**, 1-146.
- WASSERSUG, R. J., PAUL, R. D. & FEDER, M. E. (1981). Cardiorespiratory synchrony in anuran larvae (*Xenopus laevis*, *Pachymedusa dacnicolor*, and *Rana berlandieri*). *Comp. Biochem. Physiol.* **70A**, 329-334.
- WASSERSUG, R. J. & SEIBERT, E. A. (1975). Behavioral responses of amphibian larvae to variation in dissolved oxygen. *Copeia* **1975**, 87-103.
- WASSERSUG, R. J. & SPERRY, D. (1977). The relation of locomotion to differential predation on *Pseudacris triseriata* (Anura: Hylidae). *Ecology* **58**, 830-839.
- WEBB, P. W. (1975). Hydrodynamics and energetics of fish propulsion. *Bull. Fish. Res. Bd Can.* **190**, 1-158.
- WEBB, P. W. (1977). Effects of median-fin amputation on fast-start performance of rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **68**, 123-135.
- WEBB, P. W. (1981). Locomotor patterns in the evolution of actinopterygian fishes. *Am. Zool.* **22**, 329-342.
- WEST, N. H. & BURGGREN, W. W. (1982). Gill and lung ventilatory responses to steady-state aquatic hypoxia and hyperoxia in the bullfrog tadpole (*Rana catesbeiana*). *Respir. Physiol.* **47**, 165-176.