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FUNCTIONAL CONFLICTS BETWEEN FEEDING AND GAS EXCHANGE IN SUSPENSION-FEEDING TADPOLES, *XENOPUS LAEVIS*

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SUMMARY

Air-breathing tadpoles of *Xenopus laevis* (Amphibia: Anura) use buccopharyngeal surfaces for both gas exchange and capture of food particles in the water. In dense food suspensions, tadpoles decrease ventilation of the buccopharynx and increase air breathing. The lung ventilatory frequency is elevated even though the rate of oxygen consumption is at or below resting levels, suggesting that the lung hyperventilation reflects compensation for decreased buccopharyngeal respiration rather than an increased metabolic requirement. If tadpoles in hypoxic water are prevented from breathing air, they increase buccopharyngeal respiration at the expense of feeding. Aerial respiration evidently permits the buccopharyngeal surfaces to be used primarily for food entrapment.

INTRODUCTION

The gills and associated respiratory pumps of many aquatic vertebrates either function directly in feeding or cease normal operation temporarily when feeding occurs (Lauder, 1984). Thus, feeding and respiration in the buccopharynx may pose a 'functional conflict' (*sensu* Maiorana, 1977): buccopharyngeal function that maximizes food intake may inhibit gas exchange, and *vice versa* (Liem, 1980). This functional conflict is often inconsequential, for most aquatic vertebrates either feed episodically (for <5 s) or do not capture food on respiratory surfaces (Lauder, 1983).

Key words: Gas exchange, ingestion, tadpole.

However, many anuran larvae are suspension feeders in which feeding is continuous, the buccopharyngeal respiratory surfaces are the site of food particle entrapment, and buccopharyngeal ventilation is reduced in concentrated food suspensions (see below). Collection of food and mucus on buccopharyngeal surfaces must retard gas exchange (Shephard, 1981; Lauder, 1984). One potential resolution of this conflict in anuran larvae is to cease suspension-feeding when respiratory demands become acute. Another is to switch to an alternative mode of respiration (e.g. aerial gas exchange), and to maximize the trophic function of the buccopharynx at the expense of respiration. We report here the occurrence of both potential resolutions in tadpoles of the clawed frog, *Xenopus laevis* Daudin.

Xenopus tadpoles are obligate mid-water suspension feeders that capture food particles in mucus secreted from the floor of the mouth and on elaborate gill filters (Wassersug, 1972, 1980; Wassersug & Rosenberg, 1979). These tadpoles usually breathe air, with the lungs accounting for approximately 25 % of oxygen uptake in normoxic water and 100 % in hypoxic water (Feder, 1981; Feder & Wassersug, 1984). Like other amphibian larvae, *Xenopus* tadpoles can pump water through the pharynx for respiration (Wassersug, 1980; Feder & Wassersug, 1983). Unlike other larvae, *Xenopus* tadpoles lack true filamentous gills (also termed 'gill filaments') on the gill bars. However, both the gill filters and other buccopharyngeal surfaces are well-vascularized and may participate in gas exchange (Wassersug, 1972; Feder & Wassersug, 1984). For simplicity's sake we refer to the ventilation of all of these surfaces as gill ventilation. Branchial and cutaneous oxygen uptake have not been partitioned for *Xenopus* larvae, but in tadpoles of ranid frogs, these proportions range between 10–40 % and 50–80 %, respectively (Burggren & West, 1982; Burggren, Feder & Pinder, 1983). *Xenopus* larvae can meet routine respiratory needs without breathing air and will tolerate long periods (>1 week) of confinement under water (Feder & Wassersug, 1984).

In water with high concentrations of particulate matter, *Xenopus* larvae decrease gill ventilation in proportion to particle concentration (Wassersug & Hoff, 1979; Seale, 1982; Seale, Hoff & Wassersug, 1982). This pattern maintains constant ingestion rates but decreases the respiratory flow of water. Accordingly, our expectation was that larvae would increase lung ventilation in proportion to particle concentration. Conversely, curtailment of aerial respiration might compel larvae to use buccopharyngeal surfaces primarily in respiration at the expense of feeding, especially in respiratory stress. We demonstrated this experimentally by eliminating tadpoles' access to air.

MATERIALS AND METHODS

All larvae were reared from eggs and fed yeast suspensions daily. Tadpoles were maintained in aerated, aged tapwater at 25 °C on a LD 14: 10 photoperiod centred at 13.00 local time. All measurements of oxygen consumption, respiratory frequencies, and ingestion rates were made under normal laboratory illumination between 11.00 and 16.00. Experimental animals were at developmental stages 27–38 (Gosner, 1960).

We observed the respiratory frequencies of *Xenopus* larvae in various concentrations of suspended killed yeast (*Saccharomyces cerevisiae*). Respiratory moveme

[illegible]ere observed visually and counted during a timed interval to calculate respiratory frequencies. The presence of an observer had no obvious effect on the larvae. Larvae were placed individually in 1 l Erlenmeyer flasks filled with normoxic water. After 3 h acclimation, the lung ventilatory frequency was determined by timing the interval between successive air breaths for approximately 1 h. Thereafter, suspensions of killed yeast were added to the flasks, the animals were left undisturbed for 1 h, and the lung ventilatory frequency determined during the succeeding hour. The yeast added to flasks was commercial yeast (Fleischmann's Active Dry Yeast, Standard Brands, N.Y.) that had been suspended in aged tapwater, killed by boiling, and cooled. Reported yeast concentrations were either calculated from the amount of yeast added to flasks, corrected for settling (Seale *et al.* 1982), or determined directly from the optical density of yeast suspensions at 550 nm.

Gill (i.e. buccopharyngeal) ventilation frequencies were determined for nine of the larvae used in the lung ventilation measurements, and for three additional larvae at 16 mg l^{-1} suspended yeast. These frequencies were determined by visually counting the number of buccal pump strokes during a timed interval.

Rates of oxygen consumption were measured for tadpoles in Erlenmeyer flasks according to the method of Feder (1982). Killed yeast was added 1 h before measurements. Reported concentrations are the geometric mean of yeast concentrations (determined by optical density) at the beginning and end of measurements.

To measure the effects of air breathing and hypoxia on ingestion rates, groups of 14 tadpoles were assigned randomly to each of four treatments in a 2×2 design: normoxic water *vs* hypoxic water, and access to air *vs* no access to air. Groups were placed in screen cubes (8·8 cm on a side) within two larger aquaria ($23 \times 12 \times 15$ cm), one filled with normoxic water ($P_{O_2} = 150$ Torr) and one filled with hypoxic water ($P_{O_2} = 45$–50 Torr). In each aquarium, a screen was placed just below the water's surface in one cube so that tadpoles were unable to surface for air. All groups were exposed to equal suspensions of ^{14}C-labelled algae (*Chlorella pyrenoidosa*) for 1 h, during which the lung and gill ventilatory frequencies also were determined visually as described above.

Before beginning these measurements, all larvae were placed in the screen cubes in continuously aerated water on the previous evening; all had access to air. Water contained *Chlorella* (approximately 100 μg dry mass ml^{-1}) and was pumped continuously between the aquaria. On the next morning, the screens preventing access to air were put in place where appropriate, and unlabelled *Chlorella* was added to yield algal concentrations of 118–145 μg ml^{-1} in the aquaria. Algal concentrations were monitored with an electronic particle counter (Particle Data, Inc., Elmhurst, IL), and later converted to dry biomass using aliquots filtered onto pre-weighed polycarbonate filters, dried, and weighed with a Cahn electrobalance. Two hours later, pumping between the aquaria was halted, and the P_{O_2} of one aquarium was reduced to 45–50 Torr by bubbling argon through the water. Oxygen concentrations were monitored regularly with a YSI 54A O_2 meter and polarographic electrode (Yellow Springs Instrument Co., Antioch, OH). The ^{14}C-labelled algae were added 2·5 h later, resulting in a final algal concentration of 152–153 μg ml^{-1}. Animals were killed, measured, and prepared for scintillation counting after an additional hour, as [illegible]scribed below.

The algae were grown in chemostat culture on a minimal salts medium accordi to Boraas (1983), and collected from the chemostat in an overflow vessel for about 5 days. This suspension was gassed with argon, and about 1 mCi of $Na_2{}^{14}CO_3$ in culture medium was added. The suspension was then placed on a shaker table under 'cool-white' fluorescent lights for 2 days, during which 98 % of the label was incorporated. Radiocarbon content was determined in a Beckman LS-355 liquid scintillation system, with Aquasol (New England Nuclear) as the fluor. Counting efficiency was determined by the external standards method, and corrected to disintegrations per minute with a standard series of quenched samples. Tadpoles were digested completely in 1 ml of NCS solubilizer (Amersham) within glass scintillation vials before addition of the Aquasol.

RESULTS AND DISCUSSION

The amount of yeast suspended in the water clearly affected the lung ventilatory frequency (Fig. 1). The lung ventilatory frequency of larvae in a yeast suspension of 43 mg l^{-1} was not significantly different from that of larvae in water to which no yeast had been added ($P > 0{\cdot}05$; t-test for paired comparisons). However, the lung ventilatory frequency increased by 70 % ($P < 0{\cdot}05$), 159 % ($P < 0{\cdot}01$) and 888 % ($P < 0{\cdot}0001$) for larvae at 86, 173 and 269 mg l^{-1}, respectively.

The aquatic P_{O2} during these measurements was much greater than the P_{O2} at which the larvae of *Xenopus* normally increase lung ventilation in response to hypoxia (Feder & Wassersug, 1984). Thus, aquatic hypoxia *per se* is an inadequate explanation for the change in lung ventilatory frequency. Moreover, two additional measurements suggest that the lung hyperventilation described above reflects reduced aquatic gas exchange rather than the metabolic demands of processing dense food suspensions. Both the gill ventilatory frequency and the rate of oxygen consumption were inversely related to yeast concentration (Fig. 1). In contrast, the lung ventilatory frequency increased with yeast concentration. Thus, in concentrated yeast suspensions the lung ventilatory frequency was at the highest level observed, even though both the gill ventilatory frequency and the rate of oxygen consumption were low.

Because *Xenopus* larvae can respire *via* both aerial and aquatic gas exchangers, our expectation was that preventing aerial respiration should increase reliance upon aquatic gas exchange (see also Feder & Wassersug, 1984). An increased reliance upon aquatic gas exchange might compel larvae to subordinate the trophic function of the buccopharynx to its respiratory function, and even to curtail feeding. To test these expectations, we measured ingestion rate and respiratory frequencies in normoxic and hypoxic water for larvae with and without access to air at the water's surface. In hypoxic water, the larvae without access to air ingested little algae (Fig. 2). Ingestion rates were approximately 50 times greater in air-breathing larvae in the same hypoxic aquarium (Table 1). Insofar as air breathing prevents a depression in ingestion rate, air breathing facilitates feeding in *Xenopus* larvae in hypoxic water. The low ingestion of the exclusively water-breathing larvae clearly was not due to reduced gill ventilation, for the gill ventilatory rate of these larvae was 146 % of the rate of air-breathing larvae in hypoxic water (Table 1). In normoxic water, both larvae with and without access to air had high ingestion rates. Ingestion rates of the two normoxic groups ar

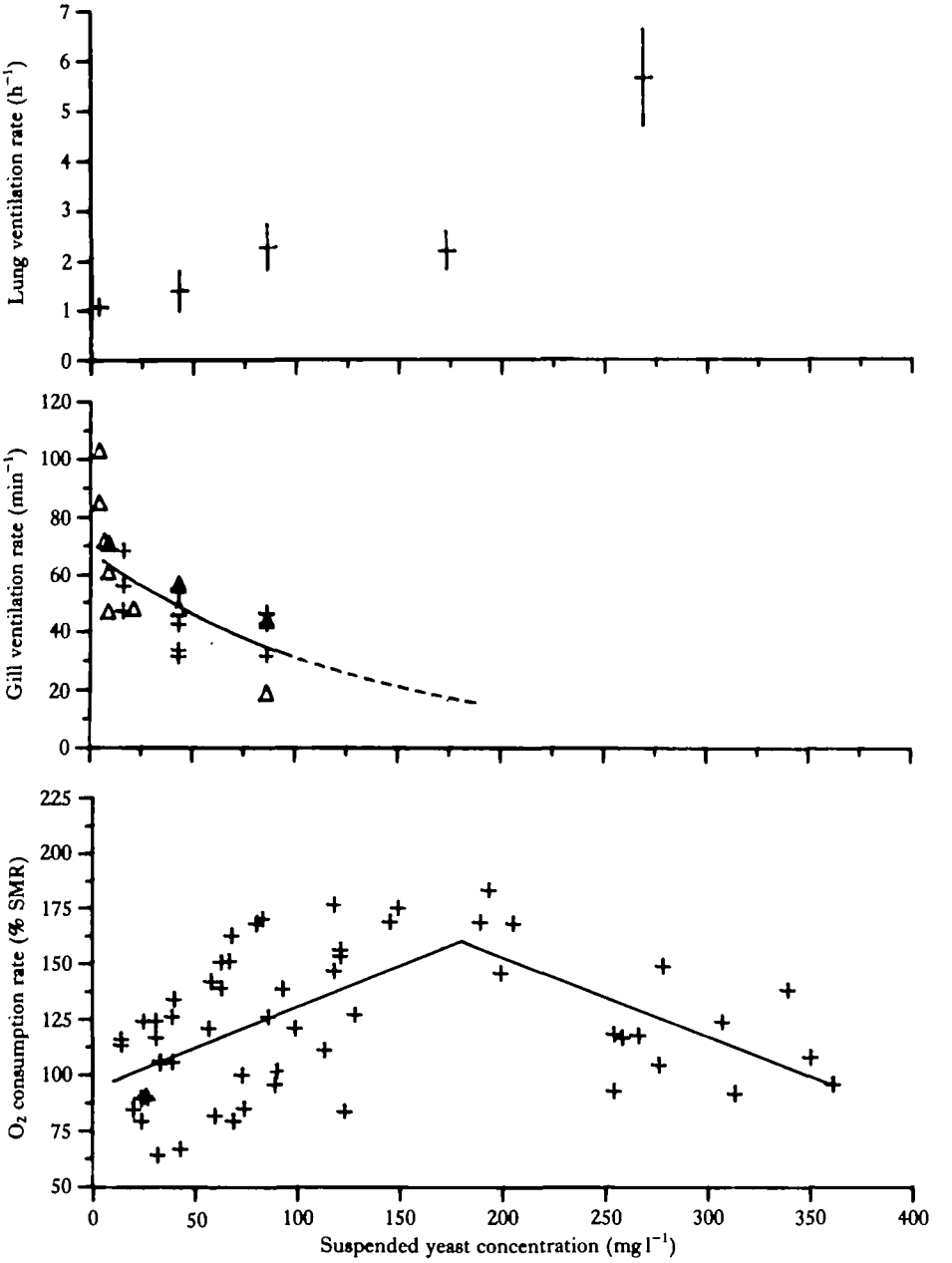

Fig. 1. Effect of suspended yeast concentration on lung (top) and gill (centre) ventilatory rates and on the rate of oxygen consumption (bottom) of *Xenopus* larvae. For lung ventilatory rates, the horizontal line indicates the mean and the vertical line indicates ± one standard error. Each larva was observed before and 1 h after addition of yeast. The symbol plotted at the lowest concentration represents the grand mean for 36 larvae before addition of yeast. The data plotted at other concentrations are for groups of nine larvae after addition of yeast. Lung ventilatory rates differed before and after the addition of yeast at all concentrations except 43 $mg\,l^{-1}$; see text. Gill (i.e. buccopharyngeal) ventilatory rates are for larvae used in measurements of lung ventilatory rates (+) and the 'discrete' values of Seale, Hoff & Wassersug (1982), Fig. 1 (△). All data were fitted to an exponential curve; the dashed line indicates extrapolation of this curve. Metabolic data are plotted as a percentage of the standard metabolic rate (SMR) expected for *Xenopus* larvae in water to which no yeast has been added (Feder, 1982). The data were fitted to two line segments (Welch, 1978).

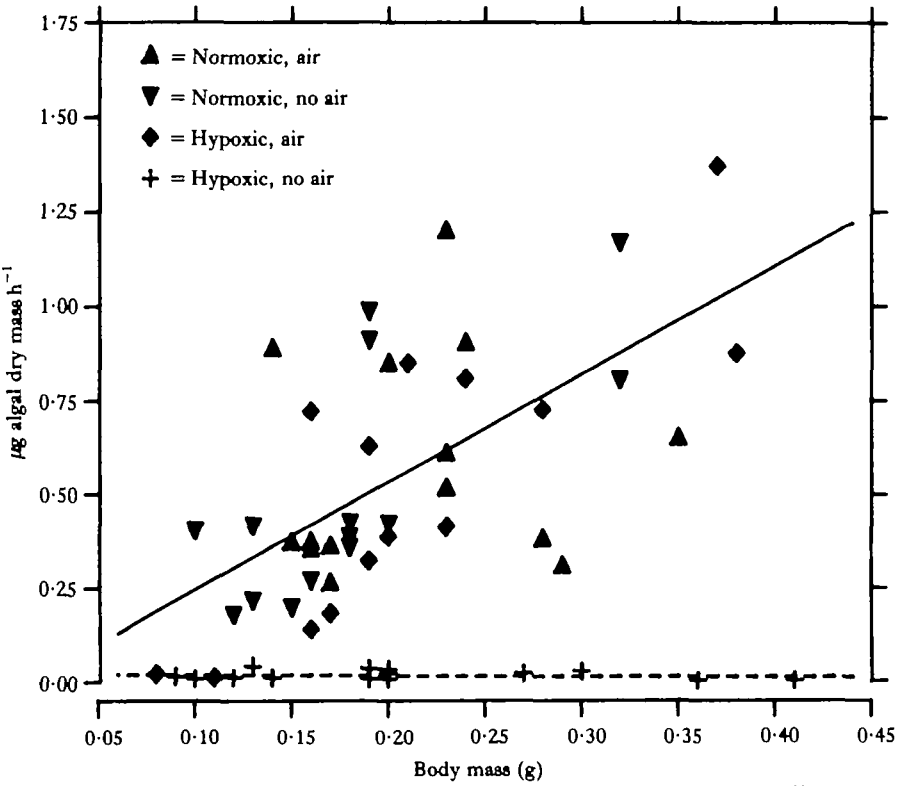

Fig. 2. Effect of body size, access to air, and aquatic oxygen tension on ingestion rates of ^{14}C-labelled algae (*Chlorella*). Values are for tadpoles after 1 h of feeding. The dashed line is the linear regression for hypoxic, exclusively water-breathing larvae. All other treatments are represented by the solid line, and did not differ significantly.

Table 1. *Effect of access to air and aquatic oxygen tension on respiratory frequencies and ingestion of ^{14}C-labelled algae*

	Access to air		No access to air	
	Normoxic	Hypoxic	Normoxic	Hypoxic
Lung ventilatory frequency (h^{-1})	4·0	30·8	–	–
Gill ventilatory frequency (min^{-1})	35·3 ± 2·5 (10)	49·5 ± 3·9 (10)	37·6 ± 2·5 (12)	72·3 ± 3·7 (16)
Ingestion rate (μg dry mass h^{-1} adjusted mean)	0·558 (14)	0·518 (14)	0·550 (14)	0·011 (14)

Means are given ± standard error; sample size is in parentheses.

the hypoxic air-breathing group did not differ significantly ($P = 0·8$; analysis of covariance), but all were significantly greater ($P < 0·0001$) than the rates of the hypoxic tadpoles without access to air. The gill ventilatory frequency was similar in normoxic larvae with and without access to air.

The decrease in food capture with a simultaneous increase in gill ventilation frequency in hypoxic, exclusively water-breathing larvae is unusual, for anuran lar

thought to regulate ingestion rates solely by modulating buccopharyngeal ventilation (Seale & Wassersug, 1979; Seale *et al.* 1982). The mechanism of this uncoupling of ventilation and ingestion is unknown, but presumably involves reducing buccopharyngeal mucous secretion. In *Xenopus* tadpoles, mucous secretion appears to vary directly with the concentration of suspended matter, and should be high at the food concentrations of the present study (Seale *et al.* 1982). A mucous layer would decrease the diffusive conductance of the branchial epithelia (Shephard, 1981). Particulate matter in the mucus would further reduce conductance, and if clumped could divert water flow from the respiratory surfaces. A decrease in secretion of mucus by the buccopharynx might eliminate each of these difficulties.

The significance of air-breathing to aquatic vertebrates extends far beyond its function in hypoxia tolerance. Lungs or other aerial gas exchangers play important roles in audition, buoyancy regulation, locomotion, growth, predator defence, reproduction (Feder & Wassersug, 1984; Kramer, 1983), and, as the present study demonstrates, feeding. Lungs appear early in the evolution of fishes (Randall, Burggren, Farrell & Haswell, 1981). As suggested by our experiments, the origin of aerial respiration may have allowed early vertebrates to commit buccopharyngeal structures to food capture (Liem, 1980).

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