J. exp. Biol. **166**, 237–251 (1992)
Printed in Great Britain © *The Company of Biologists Limited 1992*

HYPOXIC BOUNDARY LAYERS SURROUNDING SKIN-BREATHING AQUATIC AMPHIBIANS: OCCURRENCE, CONSEQUENCES AND ORGANISMAL RESPONSES

BY MARTIN E. FEDER AND DAVID T. BOOTH

Department of Organismal Biology and Anatomy and The Committee on Evolutionary Biology, The University of Chicago, 1025 East 57th Street, Chicago, IL 60637, USA

Accepted 9 January 1992

Summary

We have used oxygen microelectrodes to characterize the relationship between the partial pressure of oxygen (P_{O_2}) and the distance from the skin in a variety of amphibians immersed in unstirred normoxic water. A substantial hypoxic boundary layer surrounded the skin in nearly every case, with the P_{O_2} at the skin–medium interface typically varying between 2 and 4 kPa at both 10 and 20 °C. The degree of hypoxia varied little among the anatomical sites examined.

We hypothesized that the formation of hypoxic boundary layers should abbreviate voluntary diving in amphibians that use cutaneous oxygen uptake from water to supplement oxygen stores in the body during dives. To test this, we compared voluntary submergence times of amphibians diving in stirred and unstirred normoxic water. The dives of frogs (*Xenopus laevis*) and salamanders (*Siren lacertina*) in stirred water averaged 2.3–2.5 times longer than dives in unstirred water. Diving *Xenopus* also underwent more voluntary movements (other than swimming to the surface to breathe air) in unstirred water than in moving water. A closed extracorporeal loop containing an oxygen electrode was used to record the P_{O_2} of lung gas during forced dives in *Xenopus* to determine the time required to deplete pulmonary oxygen stores to a level normally associated with the end of voluntary dives (P_{O_2}=4 kPa). In normoxic water flowing at 0.54 cm s^{-1} or faster, the decline in pulmonary P_{O_2} to this level required on average 18.7 min; in unstirred normoxic water, the average time was 13.8 min. This difference is tantamount to a 36 % extension of submergence time in flowing water.

These findings and those of companion studies suggest that hypoxic boundary layers have a major and pervasive influence on the respiratory status of skin-breathing amphibians in water.

Introduction

Aquatic skin-breathing vertebrates must surmount a dual barrier to oxygen uptake from the medium. Oxygen must not only diffuse through the tissue

Key words: boundary layer, oxygen uptake, amphibian, ventilation, cutaneous gas exchange.

overlying the cutaneous capillaries but must also cross a layer of poorly stirred or unstirred water immediately next to the skin (Vogel, 1983; Feder and Burggren, 1985; Feder and Pinder, 1988). Indeed, the resistance of this boundary layer to gas exchange can exceed that of the skin by an order of magnitude (Pinder and Feder, 1990; Booth and Feder, 1991). Because the thickness of the boundary layer is proportional to water speed$^{-0.5}$ (Vogel, 1983; Pinder and Feder, 1990), both the ambient flow regime and any movement of the skin should affect cutaneous gas exchange, especially at low water speeds. Accordingly, the magnitude of the hypoxic boundary layer and its relationship to the ambient flow regime are physiologically significant both to organisms in which the skin is the sole or primary gas exchanger and to those in which the skin may critically supplement total gas exchange (Guimond and Hutchison, 1973; Hutchison *et al.* 1976; Boutilier and Toews, 1981; Liem, 1981; Burggren and Feder, 1986; Pinder and Burggren, 1986; Pinder and Feder, 1990; Booth and Feder, 1991).

The foregoing investigations, which have established the feasibility and importance of ventilatory regulation of cutaneous gas exchange, have with reason mostly focused on species and circumstances in which cutaneous gas exchange ought to be especially critical (e.g. frogs diving in high-altitude lakes or overwintering under water; the largest lungless salamanders; experimental designs in which animals could not deploy normal behavioural responses). To complement these previous studies and to investigate the importance of skin ventilation in less-extreme circumstances, we examined hypoxic boundary layers and their consequences in amphibian species and experimental conditions representative of the bulk of research on the environmental physiology of amphibians (Feder, 1992). We asked three questions in this context. (1) How commonplace and how severe are hypoxic boundary layers in aquatic amphibians? (2) In a diving frog, how does the movement of water outside the skin influence the rate at which pulmonary oxygen stores are depleted? (3) If hypoxic boundary layers are of consequence to typical skin-breathing amphibians, can and do such animals deploy behavioural mechanisms that mitigate these consequences? The results of the present study suggest that, amongst amphibians in general as well as in extreme situations, hypoxic boundary layers are a severe challenge to cutaneous oxygen uptake from water. Both physiological and environmental studies of cutaneous gas exchange must account for the influence of these boundary layers.

Materials and methods

Animals

Plethodontid salamanders [*Eurycea bislineata* (Green), *Gyrinophilus porphyriticus* (Green) and *Desmognathus quadramaculatus* (Holbrook)] were obtained from Amphibians of North America, Inc., Nashville, TN, USA. Frogs, *Xenopus laevis*, were obtained from NASCO, Fort Atkinson, WI, USA. *Siren lacertina* Linné (Caudata: Sirenidae) were collected near Gainesville, FL, USA. These species routinely, if not exclusively, live in water. The plethodontid salamanders

were maintained in air in plastic boxes lined with moist paper towelling and fed live insects. Frogs and *Siren* were maintained in normoxic water at room temperature and were fed commercial 'frog brittle' and cat food, respectively. All animals thrived on these regimes.

Boundary layer measurements in unstirred normoxic water

Animals were anaesthetized in 1:1000 MS-222 (tricaine methanesulphonate, adjusted to neutral pH) and tied to a plastic grid. The grid was mounted in 1:5000 MS-222 in normoxic water so that the animal's skin was approximately 3 cm below the surface of the solution. The P_{O_2} (partial pressure of oxygen) of the solution was measured with a Diamond General (Ann Arbor, MI, USA) model 737 microelectrode (250 μm diameter tip with a 3–8 μm measuring surface) mounted on a micromanipulator and connected to a Diamond General Chemical Microsensor. Between measurements and calibration of the electrode, the bath was stirred and aerated continuously. Before each series of measurements, the electrode was calibrated by first immersing it in N_2-equilibrated bath solution and then immersing it in the bath solution well away from the experimental animal. Preliminary measurements established that the P_{O_2} in the bath solution was uniform if measured at least 4 mm from the animal's skin. Accordingly, each series of measurements was begun by placing the electrode tip approximately 4 mm from the animal's skin. The electrode was then gradually advanced towards the skin, and the P_{O_2} was recorded at regular intervals (e.g. see Fig. 1). The electrode's progress was observed through a dissecting microscope set with the angle of view tangential to the curved surface of the skin and perpendicular to the electrode. When the electrode contacted the skin, it was gradually withdrawn and the P_{O_2} was recorded at regular intervals until no further change was observed. Repeated withdrawals and insertions of the electrode had negligible effects on electrode output and, presumably, the P_{O_2} at the electrode tip (Fig. 1A). After each series of determinations, stirring and aeration were begun again and the electrode was recalibrated according to the above procedure. All measurements were corrected for drift in the electrode's calibration; drift was assumed to be linear with respect to time. At the conclusion of measurements, animals were weighed and allowed to recover from anaesthesia.

The bath was constructed from a Styrofoam box (20 cm×15 cm×10 cm deep), with a window in one side for viewing the electrode. Bath temperature was regulated at 10 or 20 °C by circulating thermostatted water through a coil of copper tubing immersed in the bath. The entire assembly was placed on top of a magnetic stirrer for stirring between measurements.

Effect of water movement on diving and other movement

Xenopus laevis were maintained on a natural summer photoperiod. On the day before experimentation, animals were placed in a 15 cm diameter transparent acrylic pipe mounted on end and filled with water. The bottom of the pipe contained a plastic mesh grid, which separated the experimental animal from a

magnetic stirring bar. The depth of the water column above the grid (50 cm) was chosen to extend the length of dives (Shannon and Kramer, 1988) so that cutaneous supplementation of pulmonary oxygen uptake might be at a premium. The entire column was mounted on top of a magnetic stirrer, which was operated at approximately 250 revs min^{-1}, only as rapid as would not physically dislodge the animal while it rested on the bottom or elicit avoidance of the stirbar. The water was aerated inside an open-ended 2.5 cm diameter pipe, the lower end of which rested on the grid while the upper end protruded above the water's surface. This arrangement allowed for thorough aeration of the entire water column but did not physically buffet the animal. The animals were allowed to familiarize themselves with the apparatus overnight.

On the day of experimentation, the photophase began as usual at 06:00 h. At approximately 09:00 h, an observer entered the room and discontinued the aeration by remote control, taking care not to disturb the animals by rapid movement or by approaching the experimental chamber. Each animal was then subjected to one of two experimental treatments: (1) the magnetic stirrer was inactivated or (2) stirring was continued at the same rate as during the previous day and night. The order of these two treatments was alternated by animal. The observer recorded the time and nature of all animal movements. After 90 min, the observer began the alternative treatment (i.e. re-initiated or ceased stirring of the chamber) and again recorded the time and nature of all animal movements for the ensuing 90 min. Thereafter, water temperature and oxygen content were determined with a YSI model 54A oxygen/temperature meter and electrode.

The preceding procedure left *Siren lacertina* in a state of constant agitation and therefore required modification. To minimize agitation, holes were cut in a small clay flowerpot, which was inverted and placed on top of the grid. *Siren* would usually remain underneath this shelter, except when surfacing to ventilate their lungs, and would seldom leave the shelter during the photophase. Accordingly, illumination was dimmed at 18:00 h and the experimental periods were begun at 19:00 h. Experimental periods were 120 min each. To minimize disturbance, animals were videotaped and the experimenter was not physically present in the room except briefly and inconspicuously at the start of each experimental treatment.

Depletion of lung oxygen stores during forced dives in stirred and unstirred water

Each of seven *Xenopus* (mass 75–90 g) was anaesthetized in 1:1000 MS-222 (adjusted to neutral pH), and a PE 60 polyethylene cannula was inserted 7 mm into the glottis, where it was fixed with cyanoacrylate (Superglue Corp.). The lungs were then inflated and the apex of the right lung cannulated with PE 100 tubing by the method of Boutilier and Shelton (1986). Each frog was transferred to a flow tank (Vogel and LaBarbera, 1978) filled with 1:3000 MS-222 in normoxic water at 24–25 °C, where it rested on the bottom. The cannula was connected in series with a peristaltic pump (Gilson Minipuls 3), which ventilated the lungs with humidified room air at 2.0 ml min^{-1}. The lungs were kept inflated by securing the open end of

the glottal (outflowing) cannula under water at a slightly greater depth than the frog's lungs. Immediately before a forced dive, the lungs were collapsed by venting the glottal cannula to the atmosphere, and 4 ml of humidified air was injected into the lungs. Cannulae were then connected so that air flowed at $1.0\,ml\,min^{-1}$ in a continuous loop through the lungs, past an oxygen electrode (Instrumentation Laboratory 20984) at 25°C, and through the pump. The oxygen electrode was connected to a polarizing amplifier (Diamond General model 1201), the output of which was recorded on computer with a Datacqan (Sable Systems, Inc.) data acquisition system. The electrode was calibrated with humidified nitrogen and room air. Simulated dives lasted approximately 40 min. Dives were carried out in unstirred water and at flow velocities of 0.54, 0.83 and $1.0\,cm\,s^{-1}$. Flow velocity was determined by timing the movement of dye in the water several centimetres above the dorsal surface of the animal (see Pinder and Feder, 1990). The pattern of dye streamlines suggested that flow was laminar at these velocities.

After the foregoing measurements had been completed, pulmonary P_{O_2} was measured continuously in frogs in the unstirred normoxic flow chamber. After pulmonary P_{O_2} had reached a steady state (e.g. approximately 0.80 kPa after 30–40 min), frogs were exposed to 15–20 min cycles of flow at $1.0\,cm\,s^{-1}$ and no flow, while pulmonary P_{O_2} was recorded.

Results

Boundary layer measurements in unstirred normoxic water

At every site in every animal examined, the P_{O_2} of the water immediately adjacent to the skin differed markedly from that of the 'free stream' (i.e. the bulk medium well away from the animal) for animals immersed in unstirred normoxic water (Figs 1–3). The typical pattern consisted of a 1–3 mm thick layer of hypoxic water surrounding the skin, in which P_{O_2} declined monotonically in inverse proportion to distance from the skin–water interface. In some instances, however, uncontrollable convection in the otherwise unstirred bath was sufficient to distort the regular P_{O_2} profile in the boundary layer (e.g. 'Ventral end of costal groove' in Fig. 1B). The most physiologically significant aspect of this layer is likely to be the P_{O_2} at the skin–medium interface ($P_{O_{2s-m}}$), which represents the maximum 'driving force' for diffusion of oxygen through the skin and into the cutaneous capillaries. Typically, $P_{O_{2s-m}}$ ranged from 2 to 4 kPa (15–30 mmHg) for various regions of a representative animal (Fig. 1) and for various species of different body sizes at both 20°C (Fig. 2) and 10°C (Fig. 3). The median $P_{O_{2s-m}}$ was 2.4 kPa.

In view of this extreme regional hypoxia experienced by animals in unstirred normoxic water, we intentionally sought instances of less severe hypoxia. For example, an unusually high surface area:volume ratio of a particular anatomical region or an entire animal might entrain a lesser boundary layer and hence facilitate oxygen uptake. Such regions were evident but sometimes of little physiological significance. At the tip of a digit in a 9.5 g *Gyrinophilus porphyriticus* at 20°C, the $P_{O_{2s-m}}$ was approximately 11 kPa (Fig. 1B), but this region rep-

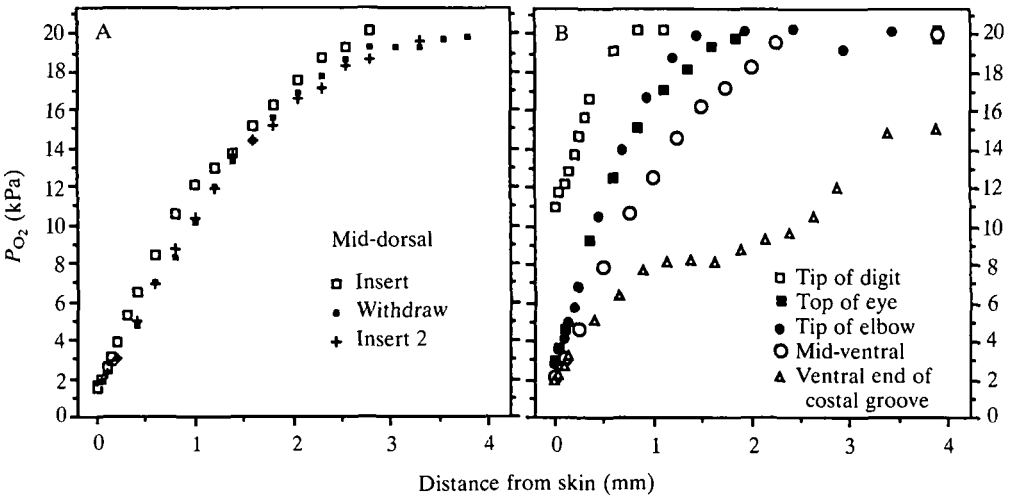

Fig. 1. Hypoxic boundary layers surrounding the skin of a single 9.5 g lungless salamander, *Gyrinophilus porphyriticus*, immersed in unstirred normoxic water at 20°C. (A) Repeatability of boundary layer measurements. P_{O_2} was measured while the oxygen microelectrode was brought towards the skin (□), withdrawn (■) and re-inserted (+). (B) Relationship between P_{O_2} and distance from the skin at varous sites on the salamander's body.

resented a negligible fraction of the entire animal's skin surface. In the relatively diminutive *Eurycea bislineata* (<1.5 g total body mass), by contrast, a considerable area of tail skin is less hypoxic (P_{O_2s-m} approximately 11 kPa) than the skin on the trunk (Fig. 2). The P_{O_2s-m} for large body parts or for large animals, however, is not exceptionally low by comparison with other measurements [e.g. compare the data in Fig. 2C (large animals) with data for smaller subjects]. Because the P_{O_2s-m} reflects both cutaneous uptake of oxygen from the boundary layer and replenishment of oxygen from the free stream, we also characterized boundary layers at 10°C, at which temperature oxygen uptake is likely to be less than at 20°C. At 10°C, however, the P_{O_2s-m} was not greater than at 20°C (Fig. 3), and in one instance was less than typical values at 20°C (Fig. 3).

Effect of water movement on voluntary diving and other movement

Both *Xenopus* and *Siren* remained at the bottom of the water column for the bulk of the observation period. Both species occasionally surfaced to breathe air. *Xenopus* exhibited several additional behaviours: frogs sometimes altered their orientation without leaving the bottom, leaned against the column's walls with the front limbs off the bottom, or swam up the water column without breaking the surface and returned to the bottom. *Siren* may well have performed such behaviours, but these animals' tendency to remain sheltered obscured them from the observer.

The P_{O_2} of the bulk medium in the water column was essentially normoxic (e.g.

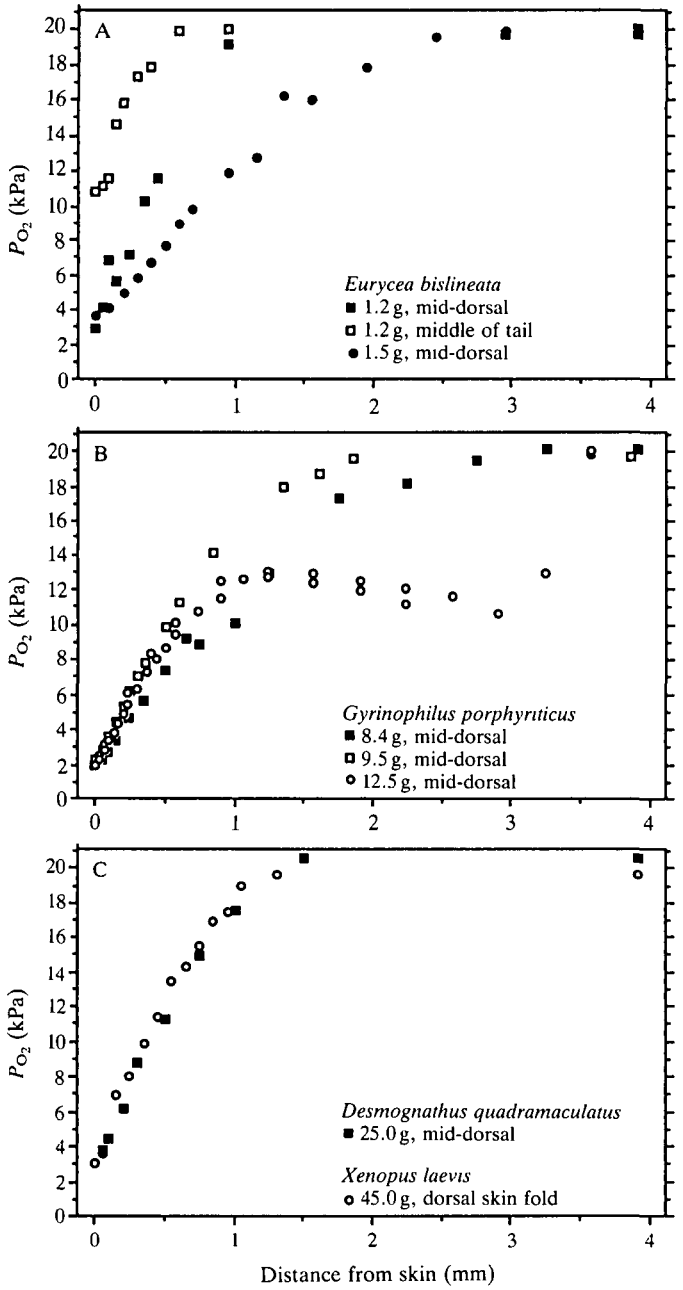

Fig. 2. Hypoxic boundary layers surrounding the skin of various exclusively skin-breathing amphibians immersed in unstirred normoxic water at 20°C. Body mass of specimen and site of measurement are indicated for each. (A) *Eurycea bislineata*, a small lungless (plethodontid) salamander that may occur in water. (B) *Gyrinophilus porphyriticus*, an aquatic lungless (plethodontid) salamander. (C) *Desmognathus quadramaculatus*, a large, aquatic lungless (plethodontid) salamander, and *Xenopus laevis*, the clawed frog (Anura: Pipidae).

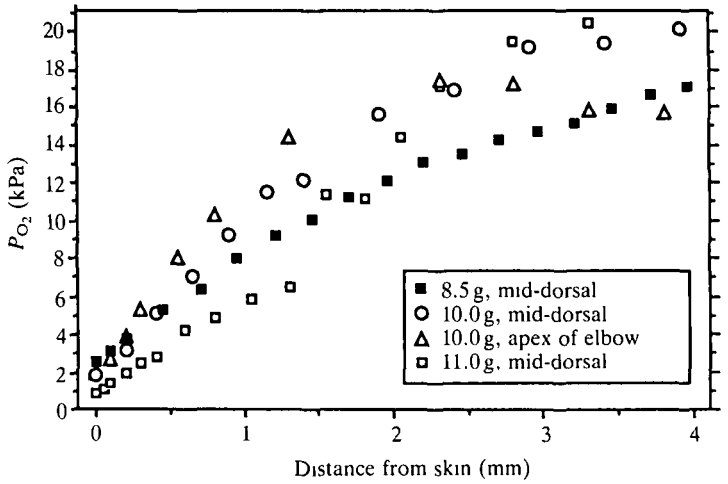

Fig. 3. Hypoxic boundary layers surrounding the skin of lungless salamanders, *Gyrinophilus porphyriticus*, immersed in unstirred water at 10°C. Plotted as in Fig. 2.

20 kPa) and homogeneous, even after intervals in which the water was unstirred. Temperatures ranged from 21 to 24°C.

The voluntary submergence times (i.e. time between air breaths at the surface) and the animals' tendencies to undergo body movements other than surfacing to breathe air varied spontaneously among individual *Xenopus* and *Siren*. Mean submergence times ranged from 4 to more than 60 min among individual *Xenopus* (Fig. 4A), and individuals' movements per hour ranged between 0 and 40 (Fig. 4C). Accordingly, the analysis followed a repeated-measures design, in which each animal's behaviour in stirred water was compared with the same individual's behaviour in unstirred water. Because our *a priori* expectation was that submergence times should be greater in stirred than in unstirred water, we calculated one-tailed P values.

In *Xenopus* (36.9–53.1 g body mass), the mean voluntary submergence time for animals in stirred normoxic water (29.2±7.5 min; ±S.E.) exceeded that for the same individuals in unstirred normoxic water (11.9±2.4 min; Wilcoxon matched-pairs signed-ranks test, $P=0.01$) and was greater in the stirred condition in 9 of 11 animals (Fig. 4A). The mean maximum voluntary submergence time was greater in stirred (45.0±7.7 min) than in unstirred water (21.8±2.6 min; Wilcoxon matched-pairs signed-ranks test, $P=0.02$), and maximum submergence durations were greater in the stirred condition in 7 of 11 animals (Fig. 4B). Differences in individuals' submergence times in stirred and unstirred water were correlated with the mean submergence time in stirred water (Spearman's $r=0.95$; $P<0.005$); i.e. stirring of the water appeared to prolong dives greatly in individuals that undertook relatively lengthy dives in unstirred water, but had a lesser effect in individuals that undertook only relatively brief dives in unstirred water.

Body movements other than those associated with surfacing to breathe air were

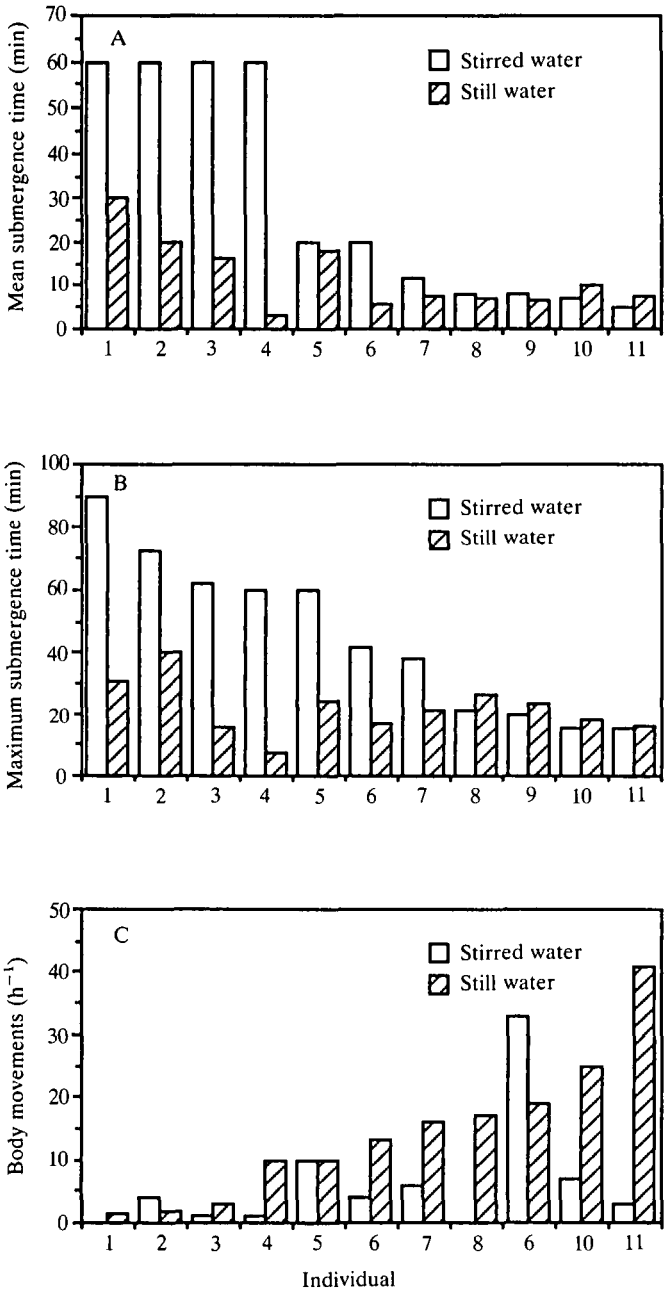

Fig. 4. Dive duration and body movements of *Xenopus laevis* diving in stirred and unstirred normoxic water. Data are for 11 individuals. For each variable, individual measurements are plotted in rank order; individual rankings are not consistent in all three graphs. In A, mean dive time is plotted as 60 min for animals taking a single air breath during the 90 min observation period.

more frequent in *Xenopus* in unstirred water ($14.3\pm3.5\,h^{-1}$) than in the same individuals in stirred water ($6.3\pm2.8\,h^{-1}$) and were more frequent in the stirred condition in 8 of 11 animals (Fig. 4C). This difference is statistically significant (Wilcoxon matched-pairs signed-ranks test, $P=0.038$). The frequency of these body movements and the durations of individuals' submergence in still water were not correlated; if routine body movements can prolong submergence in unstirred water by dissipating the boundary layer, we were unable to detect this effect.

In nine *Siren* (5.5–10.2 g body mass), individuals in unstirred water surfaced to breathe every 10.8±1.9 min (mean±S.E.), and the same individuals in stirred water averaged 42.3±11.9 min between breaths. This difference is significant (Wilcoxon matched-pairs signed-ranks test, $P=0.007$).

Depletion of lung oxygen stores during forced dives in stirred and unstirred water

In anaesthetized frogs ventilated continuously with room air, pulmonary P_{O_2} ranged from 16 to 18 kPa. Pulmonary P_{O_2} declined during forced dives (Fig. 5). Because frogs diving voluntarily typically begin dives at 16 kPa pulmonary P_{O_2} and end them at P_{O_2} values averaging 4 kPa (Boutilier and Shelton, 1986), we assessed the kinetics of this decline as the time required for the pulmonary P_{O_2} to drop from 16 to 4 kPa (Table 1). A two-way mixed-model analysis of variance with flow velocity as a fixed term and individual frogs as the random factor indicated that flow regime had a significant ($P<0.001$) effect on time for oxygen depletion. Times in unstirred water were significantly less (13.8±1.4 min; mean±S.E.; $P<0.05$) than in flowing water (18.7±1.1 min), but times at 1.0, 0.83 and $0.54\,cm\,s^{-1}$ did not differ significantly from each other ($P>0.05$, Tukey's studentized range).

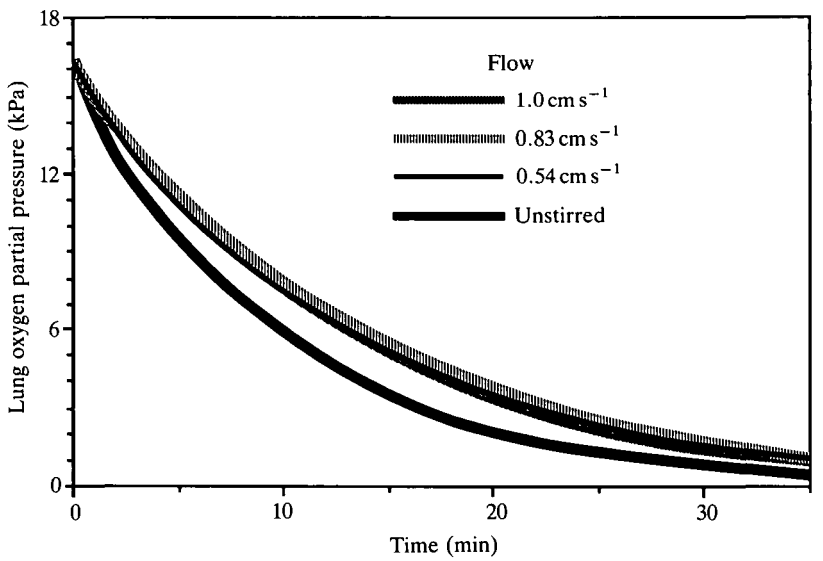

Fig. 5. Mean pulmonary P_{O_2} for the clawed frog *Xenopus laevis* at various water flow speeds throughout a 35 min simulated dive.

Table 1. *Mean times (min) for lung* P_{O_2} *to fall from 16 to 4 kPa for* Xenopus laevis *at different flow velocities*

	Flow velocity ($cm\,s^{-1}$)			
Frog no.	1.0	0.83	0.54	0
1	12.5	13.2	10.9	9.2
2	16.0	14.7	14.7	12.3
3	19.7	21.7	19.9	12.3
4	18.2	22.0	22.9	14.5
5	24.5	27.1	24.7	21.1
6	17.3			13.1
7	17.4			14.0
Mean	17.9	19.7	18.6	13.8

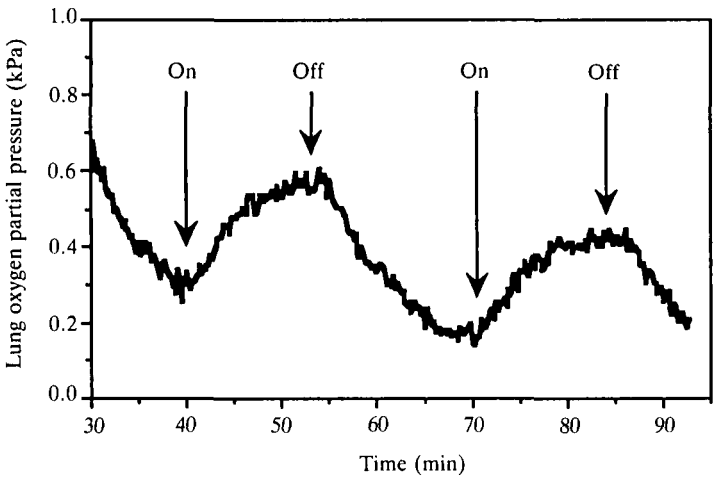

Fig. 6. Pulmonary P_{O_2} of *Xenopus laevis* during the latter part of a prolonged simulated dive in which periods of no flow were alternated with water flow at $1.0\,cm\,s^{-1}$.

After 30–40 min in the unstirred normoxic flow chamber, the pulmonary P_{O_2} of the frogs had declined to approximately 0.4 kPa. Thereafter, exposure to water flowing at $1.0\,cm\,s^{-1}$across the skin induced a 0.1–0.8 kPa increase in the P_{O_2} in the lungs (Fig. 6).

Discussion

Ecological relevance of boundary layers

The present study is the third to investigate P_{O_2} values within boundary layers surrounding various species of amphibians in various circumstances (Pinder and Feder, 1990; Booth and Feder, 1991). Several generalizations are now evident.

The respiratory medium surrounding the skin of amphibians in unstirred normoxic water is routinely hypoxic. Hypoxia is routinely severe, with P_{O_2s-m} typically 2–4 kPa. To place these values in some perspective, they are less than or equal to the P_{O_2} at the summit of Mount Everest (8848 m altitude) and less than or equal to P_{O_2} values used in experimental studies of hypoxia that were published in *The Journal of Experimental Biology* during the year 1990. Water flow past the skin can significantly, but not entirely, alleviate this hypoxia (Pinder and Feder, 1990; Booth and Feder, 1991). Accordingly, in analyses of cutaneous oxygen uptake in water, the water flow regime around the subject is likely to be at least as relevant as the gross P_{O_2} of the water, if not more so.

Are water flow rates in nature sufficiently low to pose a problem for free-ranging aquatic amphibians? Because the thickness of the fluid-dynamic boundary layer is scaled as current velocity$^{-0.5}$ (Vogel, 1983), even very slow water currents may markedly affect the thickness of the boundary layer. For an aquatic salamander, *Desmognathus quadramaculatus*, Booth and Feder (1991) estimated that current velocities in excess of 5 cm s^{-1} were necessary to maintain the P_{O_2s-m} above the critical P_{O_2} (i.e. the P_{O_2} below which standard metabolic rate is depressed by hypoxia) in normoxic water at 15 °C. In submerged anaesthetized bullfrogs (*Rana catesbeiana*) in simulated overwintering conditions (normoxic water at 5 °C), oxygen consumption declined when flow velocity decreased below 2 cm s^{-1} (Pinder and Feder, 1990). Although these laboratory determinations provide a benchmark for gauging the likely consequences of flow regimes in nature, relevant measurements of natural flow regimes are scarce. Current velocities in freshwater ponds, lakes, streams and rivers vary between 0 and 900 cm s^{-1} (Reid, 1961). In a typical tundra pond (30 m×40 m×0.5 m deep) surrounded by low vegetation, current velocities are in the range 0–1.2 cm s^{-1} when wind speeds are 200–1000 cm s^{-1} (Miller *et al.* 1980). Clearly, boundary layers will be negligible at the upper end of this range of current velocities, but substantial at the lower end of the range. Beyond this self-evident conclusion, the actual range of flow regimes experienced by amphibians in water (and the time spent in each) is almost entirely unknown. Gross current velocities reflect neither the movements of amphibians nor the effect of occupancy of underwater microhabitats sheltered from currents. In the single published study of the actual flow regime around submerged amphibians (Booth and Feder, 1991), 70 % of *Desmognathus quadramaculatus* underneath submerged rocks in a mountain stream experienced flows of 0–5 cm s^{-1}, velocities estimated to depress the standard metabolic rate. At least in this single example, even in a mountain stream skin-breathing animals may experience hypoxic boundary layers large enough to be physiologically significant (Booth and Feder, 1991). Obviously, more general conclusions must await studies that thoroughly characterize the natural flow regimes around amphibians in diverse circumstances.

Consequences of boundary layers

Because cutaneous oxygen uptake is primarily diffusion-limited (e.g. Pinder

et al. 1991), the P_{O_2s-m} is ordinarily a major determinant of the rate of cutaneous oxygen uptake. Thus, when the P_{O_2s-m} decreases below the critical P_{O_2}, cutaneous oxygen uptake will decline correspondingly (Beckenbach, 1975; Booth and Feder, 1991). Moreover, the boundary layer represents an enormous addition to the diffusive resistance of the skin alone [Feder and Pinder (1988) and Pinder and Feder (1990) give formulae for calculating diffusive resistance from the P_{O_2} in the boundary layer.] This resistance ranges from 80 % of that of the skin alone (in cold submerged bullfrogs at 5 cm s^{-1} water flow; Pinder and Feder, 1990) to more than eight times that of the skin alone (in unstirred normoxic water; Pinder and Feder, 1990; Booth and Feder, 1991; present study). Mechanisms that regulate both the diffusive and perfusive conductance of the skin to oxygen are likely to be of limited efficacy in the face of this external resistance.

For animals in which the skin is the primary or sole gas exchanger, the hypoxic boundary layer can potentially reduce the oxygen consumption of the skin itself (Vitalis, 1990), oxygen carriage to the rest of the body by the blood (Burggren and Feder, 1986; Feder and Pinder, 1988; Pinder and Feder, 1990) and the oxygen consumption of the entire organism (Booth and Feder, 1991). For amphibians with lungs that rely on pulmonary oxygen stores during dives (Boutilier, 1988), the consequences of hypoxic boundary layers, although different, are no less profound. Cutaneous oxygen uptake can conserve these stores and thereby prolong dives (Boutilier, 1988). Indeed, cutaneous uptake of oxygen from the water accounts for 14–29 % of total oxygen consumption in diving *Xenopus* (Emilio and Shelton, 1974, 1980) and 80 % of total oxygen consumption in diving *Siren* of similar size to those used in the present study (Ultsch, 1976). Hypoxic boundary layers (which reduce the P_{O_2s-m} and hence, putatively, cutaneous oxygen uptake) ought therefore to shorten dive durations. Indeed, in the present study, cessation of stirring reduced the length of voluntary dives by 59 % in *Xenopus* and 74 % in *Siren*. The influence of hypoxic boundary layers on dive duration can be related directly to their effect on the depletion of oxygen stores. *Xenopus* diving voluntarily typically begin dives at 16 kPa pulmonary P_{O_2} and end them at P_{O_2} values averaging 4 kPa, with systemic arterial P_{O_2} values at approximately 83 % of the pulmonary values (Boutilier and Shelton, 1986). In the present study, anaesthetized *Xenopus* in stirred water required 18.7 min to reduce pulmonary P_{O_2} from 16 to 4 kPa (grand mean for water flowing at 1.0, 0.83 and 0.54 cm s^{-1}). In unstirred water, by contrast, a comparable reduction in pulmonary P_{O_2} required only 13.8 min, representing a 26 % reduction in dive time. Reduction in dive time, in turn, may have major effects on evolutionary fitness (Halliday and Sweatman, 1976; Feder and Moran, 1985; Kramer, 1988).

Responses to boundary layers

There are several possible responses to the formation of boundary layers. (1) A region of the skin might become specialized in size, shape, position or roughness (Vogel, 1983; Feder and Pinder, 1988) so as to minimize the boundary layer's thickness. The measurements of P_{O_2s-m} in various regions of the skin (Figs 1–3)

suggest that this response is either absent or ineffective. Except in small amphibians or small appendages of amphibians, the P_{O_2s-m} is uniformly quite hypoxic. (2) The flow velocity of the bulk medium relative to the skin can be increased, by choice of habitats with high flow rates, by behaviour dedicated specifically to skin ventilation, or by any other movement (Vogel, 1983; Feder and Pinder, 1988). While evidence for the first of these is lacking, cutaneous ventilatory behaviour apparently occurs in at least two amphibian species (Guimond and Hutchison, 1973; Hutchison *et al.* 1976; Boutilier *et al.* 1980; Boutilier and Toews, 1981) and, in the present study, diving *Xenopus* moved more frequently in unstirred water than in stirred water. Indeed, exogenous skin ventilation can extend dive times fourfold (*Siren*, present study) and can oxygenate the lungs during a prolonged dived (Fig. 6). (3) The animal may increase reliance on non-cutaneous respiration. Both *Xenopus* (Fig. 4) and *Siren* surface more frequently to breathe air when immersed in unstirred water than in stirred water. (4) The animal may leave the water. The formation of hypoxic boundary layers is an inevitable consequence of the physical properties of water (e.g. its viscosity, O_2 capacitance and O_2 diffusivity) (Vogel, 1983; Feder and Pinder, 1988). Because of the differing physical properties of the respiratory medium, cutaneous oxygen uptake is far more practicable in air than in water. In evolutionary terms, the invasion of terrestrial habitats ought to have enhanced the capacity for cutaneous oxygen uptake by eliminating hypoxic boundary layers, provided that other transcutaneous fluxes were not compromised (Randall *et al.* 1981; Feder and Burggren, 1985).

We thank David Liefer and Byron Sebastian for patiently undertaking the behavioural observations, John Gilpin for construction of experimental apparatus and Dr Harvey Lillywhite for provision of *Siren*. Drs Randall Alberte and Rick Carlton advised us on the care and source of oxygen microelectrodes. Research was supported by NSF Grant DCB87-18264 to M.E.F.

References

BECKENBACH, A. T. (1975). Influence of body size and temperature on the critical oxygen tension of some plethodontid salamanders. *Physiol. Zool.* **48**, 338–347.

BOOTH, D. T. AND FEDER, M. E. (1991). Formation of hypoxic boundary layers and their biological implications in a skin-breathing aquatic salamander, *Desmognathus quadramaculatus*. *Physiol. Zool.* **64**, 1307–1321.

BOUTILIER, R. G. (1988). Control of arrhythmic breathing in bimodal breathers: Amphibia. *Can. J. Zool.* **66**, 6–19.

BOUTILIER, R. G., MCDONALD, D. G. AND TOEWS, D. P. (1980). The effect of enforced activity on ventilation, circulation and blood acid–base balance in the aquatic gill-less urodele, *Cryptobranchus alleganiensis*: a comparison with the semi-terrestrial *Bufo marinus*. *J. exp. Biol.* **84**, 289–302.

BOUTILIER, R. G. AND SHELTON, G. (1986). Gas exchange, storage and transport in voluntarily diving *Xenopus laevis*. *J. exp. Biol.* **126**, 133–155.

BOUTILIER, R. G. AND TOEWS, D. P. (1981). Respiratory, circulatory and acid–base adjustments to hypercapnia in a strictly aquatic and predominantly skin-breathing urodele, *Cryptobranchus alleganiensis*. *Respir. Physiol.* **46**, 177–192.

BURGGREN, W. W. AND FEDER, M. E. (1986). Effect of experimental ventilation of the skin on cutaneous gas exchange in the bullfrog. *J. exp. Biol.* **121**, 445–449.

EMILIO, M. G. AND SHELTON, G. (1974). Gas exchange and its effects on pH and bicarbonate equilibria in the blood of the amphibian, *Xenopus laevis*. *J. exp. Biol.* **83**, 253–262.

EMILIO, M. G. AND SHELTON, G. (1980). Carbon dioxide exchange and its effect on blood gas concentrations in the amphibian, *Xenopus laevis*. *J. exp. Biol.* **60**, 567–579.

FEDER, M. E. (1992). A perspective on environmental physiology of the amphibians. In *Environmental Physiology of the Amphibians* (ed. M. E. Feder and W. W. Burggren), pp. 1–6. Chicago: University of Chicago Press.

FEDER, M. E. AND BURGGREN, W. W. (1985). Cutaneous gas exchange in vertebrates: design, patterns, control, and implications. *Biol. Rev.* **60**, 1–45.

FEDER, M. E. AND MORAN, C. M. (1985). Effect of water depth on costs of aerial respiration and its alternatives in tadpoles of *Rana pipiens*. *Can. J. Zool.* **63**, 643–648.

FEDER, M. E. AND PINDER, A. W. (1988). Ventilation and its effect on 'infinite pool' exchangers. *Am. Zool.* **28**, 973–983.

GUIMOND, R. W. AND HUTCHISON, V. H. (1973). Aquatic respiration: an unusual strategy in the Hellbender *Cryptobranchus alleganiensis alleganiensis* (Daudin). *Science* **182**, 1263–1265.

HALLIDAY, T. R. AND SWEATMAN, H. P. A. (1976). To breathe or not to breathe: the newt's problem. *Anim. Behav.* **24**, 551–561.

HUTCHISON, V. H., HAINES, H. B. AND ENGBRETSON, G. (1976). Aquatic life at high altitude: respiratory adaptation in the Lake Titicaca frog, *Telmatobius culeus*. *Respir. Physiol.* **27**, 115–129.

KRAMER, D. L. (1988). The behavioral ecology of air breathing by aquatic animals. *Can. J. Zool.* **66**, 89–94.

LIEM, K. (1981). Larvae of air-breathing fishes as countercurrent flow devices in hypoxic environments. *Science* **211**, 1177–1179.

MILLER, M. C., PRENTKI, R. T. AND BARSDATE, R. J. (1980). Physics. In *Limnology of Tundra Ponds: Barrow, Alaska* (ed. J. E. Hobbie), pp. 51–75. Stroudsburg, PA: Dowden, Hutchinson & Ross, Inc.

PINDER, A., CLEMENS, D. AND FEDER, M. E. (1991). An isolated perfused frog skin preparation for the study of gas exchange. I. Effect of bulk flow on oxygen uptake and diffusing capacity. *Respir. Physiol.* **85**, 1–14.

PINDER, A. W. AND BURGGREN, W. W. (1986). Ventilation and partitioning of oxygen uptake in the frog *Rana pipiens*: effects of hypoxia and activity. *J. exp. Biol.* **126**, 453–468.

PINDER, A. W. AND FEDER, M. E. (1990). Effect of boundary layers on cutaneous gas exchange. *J. exp. Biol.* **143**, 67–80.

RANDALL, D. J., BURGGREN, W. W., FARRELL, A. P. AND HASWELL, M. S. (1981). *The Evolution of Air-breathing in Vertebrates*. Cambridge: Cambridge University Press.

REID, G. K. (1961). *Ecology of Inland Waters and Estuaries*. New York: Reinhold Publishing Corp.

SHANNON, P. AND KRAMER, D. L. (1988). Water depth alters respiratory behaviour of *Xenopus laevis*. *J. exp. Biol.* **137**, 597–602.

ULTSCH, G. R. (1976). Respiratory surface area as a factor controlling the standard rate of O_2 consumption of aquatic salamanders. *Respir. Physiol.* **26**, 357–369.

VITALIS, T. Z. (1990). Pulmonary and cutaneous oxygen uptake and oxygen consumption of isolated skin in the frog, *Rana pipiens*. *Respir. Physiol.* **81**, 391–400.

VOGEL, S. (1983). *Life in Moving Fluids*. Princeton, NJ: Princeton University Press.

VOGEL, S. AND LABARBERA, M. (1978). Simple flow tanks for research and teaching. *BioScience* **28**, 638–643.