CHARACTERIZATION OF THE INTERMITTENT BREATHING PATTERN IN XENOPUS LAEVIS

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SUMMARY

Xenopus laevis exhibits an extensive repertoire of breathing patterns during voluntary diving-emergence behaviour. In experiments where animals surfaced to breathe at a blowhole fitted with a pneumotachograph, two noticeably different patterns of breathing were observed. In the first (burst breathing), long periods of diving were periodically interrupted by short visits to the surface when a discrete series of evenly spaced ventilations occurred. On other occasions, the same animal might rise to the surface and begin ventilating its lungs, not in discrete bursts, but intermittently over a long period of time (a breathing bout). Minute ventilation during a breathing burst was more than double that of a bout and represents a more active diving-emergence behaviour on the part of the animal. Regulation of the amount of gas exchanged in both breathing styles appears to be due to manipulation of the temporal pattern of lung ventilations (i.e. the breath-hold durations), rather than to an alteration in the overall depth of breathing; the latter is possible to some extent, however, through adjustments in the composition of individual ventilations.

INTRODUCTION

Virtually all lung-breathing amphibians, whether strictly aquatic or highly terrestrial in their habits, ventilate their lungs in an intermittent fashion. These discontinuous breathing patterns result from variable duration periods of breath holding which follow each ventilation. Semiterrestrial anurans such as the bullfrog, Rana catesbeiana, and the toad, Bufo marinus, exhibit the most continuous patterns of air breathing since during the breath hold, they continue to ventilate their buccopharyngeal cavities with air (buccal oscillations; de Jongh & Gans, 1969; Macintyre & Toews, 1976; Boutilier & Toews, 1977). Unlike the bullfrog, Bufo rarely if ever engages in diving behaviour; intermittent lung ventilation with interbreath periods of buccal oscillations is the animal’s normal practice of breathing (Macintyre & Toews, 1976; Jones, 1982). Other more aquatic amphibians spend a large portion of their time under water. These animals will periodically rise to the surface, engage in an episode of breathing and then submerge. Periods between the breathing episodes
and the length of time spent at the surface are only known for a few voluntarily diving amphibians. The urodele amphibians, *Amphiuma* and *Cryptobranchus*, are noted for their prolonged periods of submergence (1–2 h) and short one or two breath emergence-submergence sequences (Toews, 1971; Toews, Shelton & Randall, 1971; Martin & Hutchison, 1979; Boutilier, McDonald & Toews, 1980; Boutilier & Toews, 1981).

In most birds and mammals, ventilation is a continuous process of rhythmic lung gas exchanges. Ventilation is closely matched with pulmonary perfusion so that the internal fluctuations of arterial blood gases and pH are kept within a comparatively narrow and well-defined range. Respiratory regulation of the internal homoeostasis of such animals is achieved through alterations in the frequency and depth of breathing. Though periods of breath holding in terrestrial mammals are usually indicative of a defective central respiratory control, these patterns would appear to be the normal output of the respiratory centre of amphibians. Certainly, the results of several studies on ventilatory responses to altered levels of inspired gases, activity and enforced diving in amphibians (see Shelton & Boutilier, 1982) all point to the conclusion that these animals possess effective respiratory control systems. Despite the number of comparative studies on intermittently breathing ectotherms (see Jackson, 1978), the factors involved in the regulation of ventilation in amphibians have been mostly confined to the periods of time spent breathing, whereas the patterns of ventilation, as possible bases of respiratory control, have received little attention.

The present study examines the intermittent breathing and diving-emergence patterns of the aquatic anuran, *Xenopus laevis*. Ventilation will be looked at from the point of view that the breath-hold intervals, in addition to respiratory frequency and depth of breathing, constitute a third major component of ventilation. In as much as this represents a behavioural component, the animals were allowed voluntarily to dive and surface under normal ambient conditions.

**MATERIALS AND METHODS**

Experiments were carried out on 17 adult female *Xenopus laevis* (120–163 g) obtained from a commercial supplier. All animals were housed in large communal tanks (20–25 °C) for several months prior to the experiments. Animals were fed chopped liver twice weekly up to 7 days before surgery.

*Animal preparation*

Arterial catheters were chronically implanted after the animals had been anaesthetized by immersion in a 0.06% solution of MS-222, buffered to pH 7. The femoral artery of one leg was occlusively cannulated in an upstream direction with a catheter prepared from Portex polypropylene tubing (P.P.50 or 60, depending on vessel size). Prior to insertion, the catheter was filled with heparinized (50 i.u. ml⁻¹) toad saline (de la Lande, Tyler & Pridmore, 1962), with 0.1 ml kg⁻¹ being injected immediately following its placement. Blood loss was invariably negligible and catheters were secured to the surrounding musculature and skin when the wound was sutured.

Following surgery, animals were placed in the thermostatted chamber (25 ± 0.5 °C) shown in Fig. 1. Their lungs were artificially ventilated until they began
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Fig. 1. Diagram of the experimental arrangement for measuring gas flowing into and out of the nares. The inverted cone in the water bath is used to guide the animal to a small cylindrical air chamber at the water–air interface. Variations in flow caused by breathing or calibrations are detected by a differential pressure transducer receiving signals from either side of a pneumotachograph screen positioned in the port which leads gases out of the air chamber. Integration of the flow profiles on either side of the 300 ml min⁻¹ baseline, gives breath volumes expired and inspired.
voluntarily to dive and surface. The toads were kept in this apparatus for the entire duration of the experiments which did not begin until at least 24 h after recovery from surgery had elapsed. Water in the tank was continuously aerated (water $P_{O_2} = 140$ Torr) and changed daily with no apparent disturbance to the animal. Humidified gases at $25 \pm 0.5 \, ^\circ C$ were introduced into the aerial portion of the chamber (volume = 15 ml) at a constant flow rate of 300 ml min$^{-1}$ (calibrated flowmeter, Rotameter Ltd, Croydon) providing complete gas renewal every 3 s. Gas flow was positioned above so that it flushed down on the nares in order to minimize the rebreathing of expired gases.

**Measurement of ventilation**

The enclosure below the breathing hole was an inverted plastic funnel. The wall of the funnel was drilled with 1 cm holes placed as close together as possible. This ensured that the changes in the water level when the animal breathed were distributed evenly over the entire surface area and not just in the breathing chamber. When *Xenopus* surfaces to breathe, it displaces only a small amount of the aerial chamber since only its snout is brought above the water line (see Brett & Shelton, 1979).

Gases passed out of the breathing chamber through a large bore plastic tubing connector which had been cut in half, fitted with a fine wire mesh (pneumotachograph screen, Fig. 1) and resealed (modified after Brett & Shelton, 1979). Holes were drilled at equal lengths on either side of the screen and inflexible nylon cannulae sealed in place so that they were flush with the inner wall. These cannulae were attached to a differential pressure transducer (Hewlett-Packard Model 270) for sensing the pressure difference across the screen. The outflow of gas from the breathing chamber was occasionally checked with a second flowmeter to ensure that it equalled the 300 ml min$^{-1}$ inflow. Calibration of the airflow was done by varying the flow rates on either side of the 300 ml min$^{-1}$ baseline. The output from the transducer was linearly related to flow over the range of flow rates encountered.

Breathing movements were detected by the transducer whose output was amplified (HP-8801A carrier preamp) and recorded continuously on tape (Racal Instrumentation) as well as hard copy (Devices chart recorder). At a later date, the tape-recorded information was fed into an analogue integrator which was calibrated with positive and negative square wave impulses for high speed replay. The output from the integrator was recorded on a fibre optics writing Medelec For-4 oscilloscope recorder, for measurement of inspiratory and expiratory volumes and durations. Breath volumes by this method were not significantly different from those obtained through digital integration by an Apple II microprocessor.

**Arterial pH, $P_{CO_2}$ and $P_{O_2}$ measurements**

Arterial $P_{CO_2}$ ($P_{mCO_2}$) and $P_{O_2}$ ($P_{mO_2}$) measurements were made by allowing blood to flow in the catheter and fill Radiometer CO$_2$ and O$_2$ electrode cuvettes. The blood displaced a 2 % CO$_2$-98 % air gas mixture into a syringe barrel connected to the outlet (Boutilier, Randall, Shelton & Toews, 1977). Following measurement (Radiometer PHM-71 display meter) blood was reinfluenced into the animal. Electrodes were frequently calibrated with humidified gas mixtures produced from a Wösthoff gas-mixing pump. All measurements and calibrations were made at $25 \pm 0.5 \, ^\circ C$. 


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pH measurements were made at 25 ± 0.5 °C by connecting the femoral cannulae to the glass capillary inlet of a Radiometer micro-pH electrode unit and coupled PHM-71 display meter. A sample of 60–80 μl was needed for each measurement and blood was then discarded. The electrode was calibrated before and after each blood sample with Radiometer precision buffers (type S1500, S1510). Haematocrit measurements were made at each sampling interval (Hawksly microcentrifuge) using approximately 50 μl of blood. Total blood loss per sample was approximately 150 μl.

**Experimental protocol**

The experiments consisted of a 24–48 h period in which each animal was allowed freely to dive and surface in air-equilibrated ambient conditions (Fig. 1). Air flow measurements from the pneumotachograph apparatus were recorded continuously throughout the experiment. At selected times, either during a breathing spell or after the animal had been submerged for 10 min or more, the arterial catheter was un-plugged and blood was allowed to flow into the electrode cuvettes and capillary tubes. Blood volume estimates of the animals used in this study ranged from 16–22 ml (using 13·4 ml 100 g⁻¹ **Xenopus** as determined by Emilio & Shelton, 1980). No more than three blood samples were taken from any one animal, which in the worst case would have resulted in less than a 3% blood loss. Haematocrit levels did decline with sampling (from 27·2 ± 0·9 to 24·6 ± 1·1; x ± 1 S.E.M.; N = 9), but such decreases, even in individual animals, would not have resulted in significant effects on blood O₂-carrying capacity (Boutilier, 1981).

Care was taken throughout the experiments to isolate the animals from outside interference, particularly during blood sampling. All observations of the animals were made from behind a blind and the entire experimental chamber was positioned on top of a thick foam pad, to reduce unwanted visual or vibratory stimuli. Blood sampling caused no apparent disturbance to the animals’ behaviour.

Mean values are presented with ± one standard error of the mean (± 1 S.E.M.). Statistical differences between mean data sets were analysed using Student’s *t*-test with *P* < 0.05 indicating a significant difference.

**RESULTS**

**Normal patterns of breathing**

**Xenopus** breathes in a highly intermittent fashion, attributable to large variations in the amount of time spent breath holding. Apart from variable duration periods of diving, the animal may exhibit one of two noticeably different patterns of intermittent breathing once it has surfaced. ‘Burst breathing’ is characterized by long periods of diving which are periodically interrupted by short surface periods when a discrete series of evenly spaced ventilations (a breathing burst) is observed (Fig. 2A). On other occasions, **Xenopus** will rise to the surface and ventilate its lungs more intermittently (unevenly spaced ventilations) over a comparatively long period of time (Fig. 2B). These prolonged sessions of breathing are defined as ‘breathing bouts’. The generic term used for bouts or bursts is a ’surface period’. During such times, the animal remains suspended at the surface with its nostrils above the water line. Non-respiratory time...
Fig. 2. Diagrammatic illustration of the two predominant breathing styles of *Xenopus laevis*. Burst breathing (A) characterized by long periods of diving breath-holds which are periodically interrupted by short surface periods when a discrete series of evenly spaced lung ventilations (a burst) takes place. Bout breathing (B) is seen when the animal spends comparatively longer periods of time suspended at the surface, ventilating its lungs much more intermittently (a bout). The non-respiratory time intervals between the breaths (surface breath-holds) are generally longer and more variable in a bout than in a burst. Periods between bursts or bouts are called diving breath-holds.
Intervals between the breaths within a surface period (burst or bout) are called surface breath-holds whereas intervals between the surface periods are times when the animal is submerged (diving breath-holds). Thus, the time intervals associated with surface and diving breath-holds dictate both the pattern and frequency of breathing.

On the other hand, the pattern of the breathing movements themselves are also variable. Though a breath always begins with a single expiration, this can be followed by one, two or three inspirations. Such differences mean that the overall breathing strategy of *Xenopus* is determined by the composition of individual breaths as well as their temporal separation.

**The composition of individual breaths**

Single ventilations were found to be of three distinct types (Fig. 3A, B, C). The most commonly occurring of these (Table 1), illustrated in Fig. 4, consisted of an exhalation which was then followed by two inhalations separated in time by an inspiratory pause. The first inhalation was continuous with the decline in the exhalant flow profile and ended when the penumotachograph flow returned to the baseline level of 300 ml min⁻¹ (i.e. baseline zero). Flow was then maintained at the baseline for a short but variable time interval (0.1–1.1 s) before the next inhalation took place. The total breath duration \( T_{\text{tot}} \) consisted of the expiratory time \( T_E \), the first inspiratory duration \( T_{I1} \), the inspiratory pause \( T_{Ip} \) and the second inspiratory duration \( T_{I2} \). The total inspiratory duration \( T_I \) was the sum of \( T_{I1} \) and \( T_{I2} \). Integration of the areas under the exhalant and inhalant flow profiles gave the expiratory volume \( V_E \) and the total inspiratory volume, \( V_I \) (where \( V_I = V_{I1} + V_{I2} \)).

Other breath patterns consisted of an exhalation followed by a single inhalation (Fig. 3B) or three inhalations (Fig. 3C; Table 1). The same measurements as shown for the double inhalation breaths (Fig. 4) were universally applied.

When triple inhalation breaths occurred (1–2% of the time, Table 1), they were most often the first breath of a surface period after a prolonged dive. This breath pattern always began with a rapid expiration, having a low \( T_E \) and a 25–40% higher peak flow rate, relative to subsequent breaths (i.e. Fig. 3C). More rapid and apparently more forceful expirations were often associated with the first breath of a surface period, regardless of whether the breath pattern was of the single, double or triple inhalation type. Though the expired volumes \( V_E \) of such breaths were highly variable, this appeared to be related to the length of the preceding dive; the longer the dive, the smaller the exhaled volume of the first breath upon emergence. The inhaled volume \( V_I \) of these rapid \( T_E \) breaths was almost always higher than that of the subsequent ventilations in the surface period. Increased inspiratory volumes occurred by several different means, including rapid \( T_I \)-high flow rate inspirations, normal \( T_I \)-high flow rates or through multiple inspirations (i.e. Fig. 3C). These high flow rate breaths are clearly visible in the burst breathing records in Fig. 6A, B and were also the characteristic breath when the animal surfaced quickly, breathed once or twice and then submerged (see below).

The end of a surface period, particularly of the burst style of breathing (i.e. Fig. 2A), was usually signalled with a low \( T_E \), low \( V_E \) expiration. Fig. 5 shows the start (panel A) and the end (panel B) of a discrete burst of double inhalation breaths. The small expiration at the end of record B was immediately followed by a dive.
Fig. 3. Representative ventilatory flow recordings of the three predominant types of breaths. Breaths always begin with a single expiration (positive flow) but may be followed by one, two or three inspirations (negative flow). Double inspiration breaths (A) are most common, followed by single inspiration breaths (B). Triple inspiration breaths (C) are comparatively rare under normal circumstances. Periods of time between the breaths represent the surface breath-holds shown in Fig. 2.
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Fig. 4. Diagrammatic illustration of double inspiration breath pattern, showing the various respiratory intervals and volume measurements made. Total breath duration (T_{tot}) is the sum of expiratory time (T_E), first inspiratory duration (T_{i1}), inspiratory pause (T_{ip}), and the second inspiratory duration (T_{i2}). The total inspiratory duration (T_I) is the sum of T_{i1} and T_{i2}. Periods between breaths are surface breath-holds. Area underneath flow profiles give expiratory volume (V_E) and inspiratory volume (V_i = V_{i1} + V_{i2}).

Fig. 5. Representative breathing records from one animal showing the start (A) and end (B) of a breathing burst under normal air-equilibrated ambient conditions. Note single expiration at end of breathing burst (B) which occurs just prior to voluntary dive.

Breath patterns

Burst breathing and bout breathing (Fig. 2) reflect two very different behaviour patterns on the part of the animal. In burst breathing, Xenopus comes to the surface for a comparatively brief period of time and ventilates its lungs at a high frequency (Figs 2A, 6A, B). Immediately following a burst, and usually after a small expiration (Fig. 5B), the animal returns to the bottom of the tank for a breath-holding period of variable duration. The overall pattern is one of discrete episodes of breathing.
Fig. 6. Representative recordings of breathing patterns in *Xenopus laevis* showing burst breathing (A, B) and bout breathing (E, F) styles. Panel C shows two bursts separated by four dives (arrows mark submergence with visual confirmation), where animal came to the surface, breathed once and then submerged. Panel D shows breath pattern during a period of time when a surface threat was imposed (see text).
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Fig. 6A, B), with the intermittent nature being related to the lengths of the dives (Fig. 2A), rather than the more consistent surface breath-holds and surface period durations. Bouts of breathing (Figs 2B, 6E, F) on the other hand, exhibit even greater amounts of variability since both the surface and diving breath-holds are irregular in their temporal patterns. Because the surface breath-holds of a bout are generally long, relative to a burst, much greater periods of time are spent at the surface for any given number of breaths. During this time, the animal remains suspended at the surface with its nares continuously exposed to the air.

Burst breathing and bout breathing were not mutually exclusive in any one animal. Spontaneous changes from one breathing style to another would occur, unannounced by any distinctive transitional pattern of breathing. Such changes sometimes took place following a dive, where a post-burst dive would be succeeded by a bout or vice versa. At other times, the distinction between the two breathing patterns was far less obvious than that shown in Fig. 6 (A, B versus E, F). For example, Fig. 6C shows two discrete bursts separated by four dives (arrows mark submergence with visual confirmation). The entire time period between the two bursts can therefore not be characterized as a diving breath-hold, since on three occasions the animal came to the surface, breathed once and then immediately submerged. These ventilations were of the rapid expiration type similar to those seen as the first breath of a surface period (Fig. 5), though never of the three inhalation pattern.

It became clear during these studies that the breathing strategy of Xenopus is closely

<table>
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<tr>
<th>Ventilatory variables within the surface periods</th>
<th>Burst breathing</th>
<th>Bout breathing</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_p$ (breaths min$^{-1}$)</td>
<td>4.98 ± 0.26</td>
<td>2.18 ± 0.65</td>
</tr>
<tr>
<td>sp Duration (min)</td>
<td>2.43 ± 0.22</td>
<td>13.56 ± 3.00</td>
</tr>
<tr>
<td>sp Breath-hold duration (min)</td>
<td>0.18 ± 0.02</td>
<td>0.44 ± 0.08</td>
</tr>
<tr>
<td>Total breath duration, $T_{tot}$ (min)</td>
<td>0.0400 ± 0.0010</td>
<td>0.0404 ± 0.0010</td>
</tr>
<tr>
<td>% Time spent breathing within sp ($f_p T_{tot}$)</td>
<td>18.9</td>
<td>8.2</td>
</tr>
<tr>
<td>Frequency occurrence of breath styles:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>single inspiration</td>
<td>0.35</td>
<td>0.36</td>
</tr>
<tr>
<td>double inspiration</td>
<td>0.64</td>
<td>0.62</td>
</tr>
<tr>
<td>triple inspiration</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Inspiratory volume, $V_{I}$ (ml)</td>
<td>10.37 ± 1.12</td>
<td>9.93 ± 1.47</td>
</tr>
<tr>
<td>$V_{I, sp}$ (ml min$^{-1}$)</td>
<td>50.91 ± 6.90</td>
<td>21.32 ± 3.93</td>
</tr>
</tbody>
</table>

Table 1. Mean values ($±1$ s.e.m.) of ventilatory variables as a function of the surface period duration

Data extracted from continuous recordings of breathing ($N = 13$) where clear distinctions between burst and bout breathing (i.e. Fig. 2) were apparent.

Number of hours of observation: for burst breathing = 27, for bout breathing = 25.5

Surface period (sp) = an episode of breathing at the surface uninterrupted by a dive (i.e. burst or bout).

$f_p$ = the frequency of breathing within a surface period.

$T_{tot}$ = the duration of a single breath consisting of the expiratory duration ($T_E$); the inspiratory duration ($T_I$) where $T_I$ may consist of one, two or three discrete inspirations (i.e. $T_{I,1}$, $T_{I,2}$, $T_{I,3}$); and the inspiratory pause duration ($T_{IP}$) where $T_{IP}$ may consist of either one or the sum of two discrete respiratory pauses (i.e. $T_{IP,1}$ or $T_{IP,2}$ and $T_{IP,3}$).

The inspiratory volume ($V_{I}$) may consist of one, two or three discrete inspirations (i.e. $V_{I,1}$, $V_{I,2}$, $V_{I,3}$).

The inspiratory ventilation rate as a function of the surface period duration ($V_{I, sp}$) = $f_p V_{I}$.

All volumes given in ml/200g animal.

Temperature = 25°C.
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Fig. 6A, B), with the intermittent nature being related to the lengths of the dives (Fig. 2A), rather than the more consistent surface breath-holds and surface period durations. Bouts of breathing (Figs 2B, 6E, F) on the other hand, exhibit even greater amounts of variability since both the surface and diving breath-holds are irregular in their temporal patterns. Because the surface breath-holds of a bout are generally long, relative to a burst, much greater periods of time are spent at the surface for any given number of breaths. During this time, the animal remains suspended at the surface with its nares continuously exposed to the air.

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<tr>
<td>Quantity</td>
</tr>
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<td>-----------------------------------------------</td>
</tr>
<tr>
<td>( t_p ) (breaths min(^{-1}))</td>
</tr>
<tr>
<td>sp Duration (min)</td>
</tr>
<tr>
<td>sp Breath-hold duration (min)</td>
</tr>
<tr>
<td>Total breath duration, ( T_{bw} ) (min)</td>
</tr>
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<tr>
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</tr>
<tr>
<td>Inspiratory volume, ( V_i ) (ml)</td>
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<tr>
<td>( V_{1,sp} ) (ml min(^{-1}))</td>
</tr>
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Data extracted from continuous recordings of breathing \((N = 13)\) where clear distinctions between burst and bout breathing (i.e. Fig. 2) were apparent.

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Surface period (sp) = an episode of breathing at the surface uninterrupted by a dive (i.e. burst or bout).

\( t_p \) = the frequency of breathing within a surface period.

\( T_{bw} \) = the duration of a single breath consisting of the expiratory duration \((T_E)\); the inspiratory duration \((T_i)\) where \( T_i \) may consist of one, two or three discrete inspirations (i.e. \( T_{i1}, T_{i2}, T_{i3} \)); and the inspiratory pause duration \((T_{ip})\) where \( T_{ip} \) may consist of either one or the sum of two discrete respiratory pauses (i.e. \( T_{ip1} \) or \( T_{ip1} + T_{ip2} \)).

The inspiratory volume \((V_i)\) may consist of one, two or three discrete inspirations (i.e. \( V_{i1}, V_{i2}, V_{i3} \)).

The inspiratory ventilation rate as a function of the surface period duration \((V_{1,sp}) = t_p V_i\).

All volumes given in ml/200g animal.

| Temperature = 25°C. |
linked with visual cues from the surface. Sudden visual or vibratory stimuli delivered during any surface period always resulted in a rapid submergence to the bottom of the tank where the animal would eventually remain quite still. If the tank was not shielded and direct observations were made from above the animal, the breathing strategy changed. During such times, (Fig. 6D) the animal would make rapid excursions to the surface, breathe quickly (i.e. low $T_E$, high peak flow rate), and then immediately return to the bottom (far left of panel D). Apparently, the animal also favoured much longer periods of submergence rather than coming to the surface (note 55 min dive, Fig. 6D).

Periodic interruptions (i.e. Fig. 6C) of an otherwise regular pattern of burst breathing (i.e. Fig. 6A, B) make strict objective criteria for distinguishing between burst and bout breathing very difficult to establish. Sufficiently long periods of observation have, however, revealed clear distinctions between the two most commonly occurring breathing styles (i.e. Fig. 6A, B versus Fig. 6E, F) and quantitative analyses were carried out for approximately 50 h of recordings from thirteen animals. Table 1 shows mean data for several respiratory variables associated with burst and bout breathing. The frequency of breathing within the surface period ($f_{sp}$) was on average two times higher for a burst than for a bout. The higher $f_{sp}$ of burst breathing occurred by virtue of shorter breath-holds and a reduction in the surface period duration. Thus, the percentage of the total surface time spent actively breathing in a burst breathing episode was twice that when the animal was engaged in bout breathing. The frequency occurrence of single, double and triple inhalation breaths and the breath duration ($T_{tot}$) were the same for both breathing styles (Table 1). So too were the mean

Table 2. Mean values (± 1 S.E.M.) of ventilatory variables as a function of the total time, including ventilatory and non-ventilatory periods

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f$ (breaths min$^{-1}$, total time)</td>
<td>0.77 ± 0.17</td>
</tr>
<tr>
<td>Breath-hold duration (surface plus diving breath-holds, min)</td>
<td>1.46 ± 0.21</td>
</tr>
<tr>
<td>Expiratory duration, $T_E$ (s)</td>
<td>0.68 ± 0.09</td>
</tr>
<tr>
<td>Inspiratory duration, $T_I$ (s)</td>
<td>0.82 ± 0.08</td>
</tr>
<tr>
<td>Total breath duration, $T_{tot}$ (s)</td>
<td>2.28 ± 0.06</td>
</tr>
<tr>
<td>Expiratory volume, $V_E$ (ml)</td>
<td>9.65 ± 1.98</td>
</tr>
<tr>
<td>Inspiratory volume, $V_I$ (ml)</td>
<td>12.44 ± 2.12</td>
</tr>
<tr>
<td>Expiratory ventilation, $V_{E'}$ (ml min$^{-1}$)</td>
<td>7.42 ± 0.61</td>
</tr>
<tr>
<td>Inspiratory ventilation, $V_{I'}$ (ml min$^{-1}$)</td>
<td>9.56 ± 0.60</td>
</tr>
<tr>
<td>Number of inspirations per expiration</td>
<td>1.62 ± 0.02</td>
</tr>
<tr>
<td>Ratio: $V_E/V_I$</td>
<td>0.77 ± 0.03</td>
</tr>
<tr>
<td>% Time spent breathing ($f$·$T_{ua}$)</td>
<td>2.9</td>
</tr>
</tbody>
</table>

$f$ = the mean overall breathing frequency.
$T_E$, $T_I$ and $T_{tot}$ ($T_E + T_I + T_{tp}$) as defined in Table 1 and text where $T_{tp}$ = inspiratory pause duration.
$V_E$ = the expiratory ventilation rate as a function of total time ($f$·$V_E$).
$V_I$ = the inspiratory ventilation rate as a function of total time ($f$·$V_I$), where $V_I = V_{I1}$, $V_{I2}$, $V_{I3}$.
Data points contributing to each mean value shown were averaged values of 2-4 h observation from one

*Xenopus* ($N = 7$).
Total number of hours observed = 27.
Ventilation volumes expressed as ml/200 g animal.
Temperature = 25°C.
Intermittent breathing in *Xenopus*

Respiratory volumes of the individual breaths. Thus, the large inspired volume per unit time ($\dot{V}_i$) of burst breathing, relative to bout breathing, was a direct reflection of the differences in $f_{up}$. Regardless of the breathing style, differences between mean inspired and expired volumes (i.e. Table 2) were to be expected on the basis of the proportionately greater skin $CO_2$ losses than $O_2$ uptake.

**Breath-hold patterns**

Respiratory frequency falls into two categories; (1) the frequency of breathing within the surface periods, denoted $f_{sp}$, and (2) the frequency of breathing as a function of the total time, denoted $f$ (Tables 1 and 2). The respiratory frequency, $f$, of *Xenopus* at 25 °C was always found to be less than 1 breath min$^{-1}$ if the observation period was long (3–5 h). Analyses over shorter time intervals increased the chances of observing more or less frequent periods of breath holding, leading to large differences in the overall respiratory rate of an animal. For example, sequential analysis of a 10 h recording in one animal gave the following results (interval observed in h, $f$ in breaths min$^{-1}$): 0–2, 1·46; 0–4, 1·01; 0–6, 0·74; 0–8, 0·77; 0–10, 0·61. These data indicate that $f$ is more likely to be overestimated if the observation period

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**Fig. 7.** (A) Frequency histogram for all breath-hold durations (surface and diving) in 65 h of recordings from eight animals. Small solid blocks at bottom are when a single breath-hold was recorded for the time interval looked at. (B) Log-survivor plot where the abscissa corresponds to a breath-hold duration of time $t$, and the log ordinate displays the percentage number of breath holds whose lengths are greater than time $t$. The curve is a plot of the frequency histogram in Fig. 7A.
is short. This seems even more probable considering that long breath-holds, being most infrequent (Fig. 7A), are likely not to be included or complete within a short observation period.

At 25 °C *Xenopus* spends only 2.9% of its time actively engaged in ventilating the lungs (Table 2). Thus, the majority of the time is spent breath holding, which may last for only a few seconds or up to an hour or more. Because the control systems involved in the onset and termination of a breath (or breath series) are very poorly understood, there is no a priori reason to treat surface and diving breath-holds as separate entities. Taken together, however, a single mean value of the surface and diving breath-hold durations (Table 2) is not a very useful parameter with which to describe intermittent breathing. A frequency histogram for all of the breath-hold durations observed in 65 h of recordings from eight animals is illustrated in Fig. 7A (at least 6 h of continuous recordings were analysed per animal). Clearly, most of the breath holds observed were those associated with the surface periods (i.e. surface breath-holds). Longer lasting breath-holds (5–60 min) represent exclusively the frequency occurrence of the time periods spent under water (diving breath-holds). To examine the breakdown of surface and diving breath-holds more closely, a log-survivor plot of the frequency histogram was constructed (Fig. 7B). In this plot, the abscissa corresponds to a breath-hold duration of time \( t \), and the log ordinate displays the percentage number of breath-holds whose lengths are greater than time \( t \). These plots are used widely for quantitative analyses of temporal patterns of behaviour (Fagen & Young, 1978), where intervals between the behavioural events are classified into short within-bout (or burst) and long between-bout (or burst) groupings.

The advantage of the log-survivor function is that it has the property of being linear when the probability of the interval ending (i.e. the next breath occurrence) is constant. Because the slope of the curve is proportional to the probability, changes in slope can be used as the bout (burst) criterion (Fagen & Young, 1978), in this case signalling the separation between the short surface breath-holds and those associated with diving (Fig. 7). The straight lines in Fig. 7B were fitted by eye where obvious breaks had occurred in the log-survivor function. The change in slope at the 1–5 min mark (Fig. 7B) represents the division between the short surface breath-holds of burst breathing and the comparatively longer and more widely ranging breath-hold durations of the bout breathing behaviour (Table 1). Taken together, these surface breath-holds make up 90% of the total number of breath-holds observed. The break at 3–5–4 min is taken to suggest that a dive is occurring. Breath-holds of between 4 and 14 min represent about 40% of the dives observed (6% of the total number of breath holds; Fig. 7A). The percentage number of breath holds of 14 min and beyond declines quite steeply (Fig. 7B) with only 10% of the dives being 25 min or more in duration (1% of the total).

The log-survivor plot therefore describes all of the surface and diving breath-hold patterns of *Xenopus*. After 1–1.5 min of breath holding, the probability of a subsequent breath becomes reduced. If, however, the breath hold duration is between 1.5 and 3.5 min, this would indicate that a breathing bout is probably taking place. Surface breath-holds during burst breathing would be represented exclusively within the 0–0.5 min duration (Fig. 7A, B). Intervals beyond 3.5–4.0 min indicate that the animal has submerged and that the probability of the next breath occurrence becomes...
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Table 3. Mean (± 1 s.e.m.) arterial blood pH, Pco₂ and P0₂ values of nine Xenopus at 25°C

<table>
<thead>
<tr>
<th></th>
<th>Breathing at surface</th>
<th>Diving</th>
</tr>
</thead>
<tbody>
<tr>
<td>pHₐ</td>
<td>7.753 ± 0.010</td>
<td>7.708 ± 0.010</td>
</tr>
<tr>
<td>Paco₂ (Torr)</td>
<td>16.9 ± 0.9</td>
<td>21.7 ± 1.0</td>
</tr>
<tr>
<td>Pao₂ (Torr)</td>
<td>84.4 ± 5.0</td>
<td>38.9 ± 4.9</td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>14</td>
</tr>
</tbody>
</table>

Blood samples taken near the suspected end of a voluntary dive range from the 12 to 20 min mark of breath holding.

its lowest for the following 10 min. Beyond 15 min of breath holding, the probability then increases as the animal begins to surface for its next breathing episode.

Blood gases and pH

Arterial blood pH, Pco₂ and P0₂ levels measured in blood samples taken whilst the animals were breathing (bursts or bouts) were similar to the values obtained by Emilio & Shelton (1974, 1980) for Xenopus at 25°C (Table 3). Blood samples taken after the animals had been voluntarily diving for 12–20 min revealed on average a small decline in pHₐ of 0.045 units, a 4.8 Torr increase in Paco₂ and a 45.5 Torr fall in arterial O₂ tension. These data collected during voluntary dives, characterize the development of a mild respiratory acidosis and progressive hypoxaemia in arterial blood. There was no reason to suspect, either from the pH-Pco₂ values or from the levels of Pao₂, that these voluntary dives were anything other than aerobic.

DISCUSSION

Though the intermittent breathing patterns of aquatic amphibians are often commented upon, there is still only a small amount of information regarding the factors which may control these spontaneous diving-emergence behaviour patterns (Toews et al. 1971; Toews, 1971; Shelton & Boutilier, 1982). Rather more information is available for the episodic breathing of reptilian species (reviewed by Wood & Lenfant, 1976) with the greatest attention having been paid to the Chelonia (Jackson, 1978; Burggren & Shelton, 1979; Milson & Jones, 1980 amongst others). Owing to the long breath-holding periods associated with diving, the respiratory rates of such animals are difficult to quantify in a way in which the nature of the breathing and non-breathing patterns can also be characterized. The results of the present study suggest that log-survivor analyses of the breath-hold durations (Fig. 7B) can provide many useful criteria with which to characterize these intermittent breathing behaviour patterns. This plot has the further advantage of being standardized, so that interspecific comparisons may be made directly. A survey of the available literature on amphibians and reptiles is under way (R. G. Boutilier & M. L. Glass).

Classification of the overall breathing patterns of Xenopus into two distinct breathing styles (Figs 2, 6; Table 1) has revealed certain interrelationships between the behavioural and physiological states of the animal. If the surface poses some threat,
burst breathing or even more rapid emergence-submergence sequences (i.e. Fig. 6I) are the ventilatory strategies which the animal is most likely to adopt. These periodic breathing episodes may also represent times when there is a behavioural requirement to be under water. Prolonged sessions of bout breathing (Figs 2B, 6E, F) seem less akin to the aquatic habits of *Xenopus*, particularly since there are no additional surface activities which are readily apparent. Additionally, gas exchange can be more rapidly achieved by a breathing burst (Table 1), providing less exposure time at the surface. Ventilatory bouts may simply represent periods of rest which occasionally interrupt the more active diving-emergence behaviour of the burst breathing style. During these prolonged surface periods the animal remains suspended at the surface with the only activity being that of the breathing movements themselves. The ventilatory requirement would therefore be less, consistent with the lower $f_{ap}$ and $V_t$ of bout breathing (Table 1). The manner in which *Xenopus* breathes during a burst (rapid and regular ventilations, Fig. 6A, B) suggests that the strategy is one of maximizing gas exchange (high $f_{ap}$, high $V_t$; Table 1) in the least amount of time. Regulation of the amount of gas exchanged in bursts and bouts appears to be controlled exclusively through a manipulation of the non-ventilatory intervals between the breaths, since the inhaled volumes ($V_t$) are the same for both breathing styles (Table 1).

Differences between resting and active levels of ventilation have also been observed in freely moving seals (Kooyman, Kerem, Campbell & Wright, 1973; Craig & Pasche, 1980). These studies on the diving mammal describe patterns of breathing which are analogous to the burst and bout breathing defined herein. Kooyman and his colleagues found that the Weddel seal would often rest at the blowhole for prolonged periods, sometimes sleeping. Like *Xenopus*, the $f_{ap}$ of the resting seals was reduced when compared to that seen during their more active diving (burst breathing) behaviour patterns. However, the seals also adjusted their depth of breathing so that the more active animal (i.e. diving-emergence behaviour) exchanged greater amounts of gas per breath. Inhalant volumes in anuran amphibians are generally thought to be limited by a constant volume buccal force pump. Air is drawn into the buccopharyngeal cavity and then forced into the lungs by contraction of the floor of the buccal cavity (glottis open, nares shut; de Jongh & Gans, 1969; West & Jones, 1975; Brett & Shelton, 1979). Though gas exchange under normal circumstances (burst and bout breathing) appears to be set by the $f_{ap}$ (i.e. surface breath holds, Table 1) there are indications that *Xenopus* can make active adjustments of the amount of gas exhaled and inhaled. The comparatively higher exhalant flow rates and lower expiratory durations of the first breath following a diving breath hold (Figs 5A, 6A) indicate a more forceful expulsion of lung gas. Brett (1980) has shown that *Xenopus* contracts its flank musculature during an expiration and suggested that this, in addition to elastic recoil and pulmonary smooth muscle effects (de Jongh & Gans, 1969; Smith & Rapson, 1977), might aid in forcing gas out of the lung. If the high flow rate expiration of the first breath served to empty the lung more completely (pre- and post-dive lung volumes may vary considerably; Shelton, 1976), this would explain the increased $V_t$ of the subsequent inspiration(s), relative to the breaths which follow (Figs 5, 6A). It would also seem a useful breathing strategy, whereby the first breath following a dive would always ensure a fast and efficient turnover of lung gases. This was clearly the breathing strategy being employed while a surface threat was imposed (Fig. 6I).
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Even rapid acquisition of fresh lung gas would aid in reducing the amount of time required at the surface. In addition, Brett & Shelton (1979) pointed out the adaptive advantages that the expiratory-inspiratory sequence of *Xenopus* (i.e., Fig. 4) must have in terms of the animal's aquatic lifestyle. This sequence ensures that the mixing of the pre-breath lung gases with freshly acquired air will be kept to a minimum, whereas this is not so clearly the case for the inspiratory-expiratory sequence of the bullfrog (de Jongh & Gans, 1969) and other more terrestrial anurans (West & Jones, 1975; Macintyre & Toews, 1976).

The flank musculature of *Xenopus* has also been implicated in the control of buoyancy (de Jongh, 1972) both during a dive and for the periods of breathing at the surface. Presumably, the expiration of gas at the end of a breathing period (Fig. 5B) means that the animal adjusts its lung volume prior to diving. This indicates that there may be lung mechanoreceptors which provide information towards establishing an optimal lung volume, allowing for negative buoyancy during the dive. The diving volumes of the more primitive lungs of urodele amphibians such as *Amphiuma* and *Cryptobranchus* are invariably adjusted 5-15 s following submergence, when air is expelled as bubbles through their spiracular openings (Toews, 1971; Boutilier & Toews, 1981). This form of adjustment was also observed in *Xenopus*.

The mammalian concept of tidal volume is not applicable to animals which respire at more than one site of gas exchange. In *Xenopus*, the amount of gas inspired is always greater than the amount expired over a series of several dives (Tables 1 and 2). This results from the comparatively greater skin CO2 losses, relative to cutaneous O2 uptake (Krogh, 1904; Hutchison, Whitford & Kohl, 1968) and reflects the large differences in the solubilities of these respiratory gases in aquatic media (Dejours, 1975). As a consequence, the lung volume of the submerged animal will decline as gas is exchanged (cf. Emilio & Shelton, 1974). In doing so, the alveolar P02 presumably declines as the animal utilizes the lung as an O2 store throughout the dive. There was no evidence from pre- and post-dive ventilation rates or blood gas measurements that a switch to anaerobic pathways was ever enforced on the animal during a voluntary dive. Considering the capacity for tissue CO2 storage and non-pulmonary CO2 losses, the small increase in P̂aCO2 during a dive, relative to the large P̂aO2 decline, may mean that there is some balance being struck between tissue CO2 output and transcutaneous CO2 elimination. The possible existence of an acid-base homoeostasis during voluntary diving, coupled with a periodic replenishment of the oxygen store, fits well with the breathing patterns observed in this and other studies (cf. Shelton & Boutilier, 1982).

There can be no doubt, however, that the behaviour of *Xenopus* (or other intermittent breathers) figures largely in these animals' regulation of ventilation. Though the intermittent nature of the breathing movements themselves are often concentrated upon, animals like *Xenopus* spend most of their life under water (i.e., Table 2). This non-respiratory time must surely represent an enormous 'ventilatory reserve' which could be called upon quite independently of changes in the volumes of individual breaths. Even though there appears to be some degree of flexibility with the positive displacement buccal pump of *Xenopus*, the major effective component of ventilation is probably manipulation of the temporal patterns of lung ventilations. In this regard, log-survivor analyses may provide useful objective criteria for control system studies of intermittent ventilation in many animal species.
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REFERENCES


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