The metabolic response to hypoxia and emersion of aestivating fishes (Lepidogalaxias salamandroides and Galaxiella nigrostriata) and a non-aestivating fish (Bostockia porosa) from south-western Australia

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Abstract
We measured the metabolic rate of three fishes (Lepidogalaxias salamandroides, Galaxiella nigrostriata, Bostockia porosa) that are endemic to the south-west of Western Australia. The first two species have been reported to aestivate, the third does not aestivate when the ponds dry up in late summer. For normoxic conditions, the metabolic rates of B. porosa and G. nigrostriata in water (0.48 mL g⁻¹ h⁻¹ and 0.44 mL g⁻¹ h⁻¹ respectively), are significantly higher than in air (0.21 mL g⁻¹ h⁻¹ and 0.08 mL g⁻¹ h⁻¹ respectively) but for the more benthic and terrestrially mobile L. salamandroides there was no significant difference between VO₂ in water (0.29 mL g⁻¹ h⁻¹) and air (0.18 mL g⁻¹ h⁻¹). Progressive hypoxia (12, 5 and 2% O₂) decreased the metabolic rate of G. nigrostriata and B. porosa in both water and air but there was a reduction in metabolic rate for L. salamandroides only in water. The metabolic physiology of L. salamandroides in water and air is consistent with the capacity to aestivate in moist soil, but the different metabolic response of G. nigrostriata suggests that it adopts a different strategy to L. salamandroides to survive when the ponds dry up in summer. The metabolism of G. nigrostriata in air and water declines with progressive hypoxia (from 12 to 5 to 2% O₂). B. porosa does not appear to be able to cope metabolically when out of water or under hypoxic conditions, and therefore would not be able to aestivate.

Introduction
Many species of fish have been reported to hibernate in response to cold, or aestivate in response to heat and dryness; aestivation is particularly common for fish that occur in ephemeral water bodies (Graham 1997). For freshwater fishes, an impetus for air-breathing has been the hypoxia of stagnating aquatic environments. The high content of decaying organic materials in the swamps of the south-west of Western Australia is likely to reduce the availability of O₂ in some of these waterways.

Of the eight endemic species of fishes in the rivers, creeks and waterways of the south-west of Western Australia (Allen 1982), at least two species, the salamanderfish (Lepidogalaxias salamandroides) and the black-striped minnow (Galaxiella nigrostriata), are thought to aestivate when the ponds and creeks dry up in summer (Pusey 1989b; Allen and Berra 1989).

The nightfish (Bostockia porosa), another endemic inhabitant of the streams and creeks of the south-west region of Western Australia, hides during the day and only emerges from its refuge at night. There is no evidence that the nightfish aestivates although it is found in the same shallow, swamps and creeks as L. salamandroides and G. nigrostriata.

Although much is known of the distribution, reproductive biology and ecology of these three species, little is known of their physiological responses to aestivation and hypoxia (Pusey 1989b; Martin et al. 1993; Berra and Allen 1995). The objectives of this study were two-fold. First, we examined the metabolic rate of L. salamandroides, G. nigrostriata and B. porosa in water and in air, to determine whether these species had metabolic adaptations related to aestivation. Second, we examined the metabolic rate of these three species under a range of hypoxia conditions.
hypoxic conditions (12, 5 and 2% O₂ concentration) to determine how these species might respond to the natural hypoxia as their ponds dry over summer (Pusey and Edward 1990).

Materials and Methods

Salamanderfish, black-striped minnow and nightfish were collected from the south-west of Western Australia during mid-January. They were acclimatised in outdoor aquaria filled with fresh water and subject to normal photoperiod for at least three weeks before being used in experiments. All experiments were conducted in water taken from the container holding the fish.

Metabolic rate was measured by flow-through respirometry. *L. salamandroides* and *G. nigrostriata* were sealed in a 25-mL clear glass flask, either in about 15 mL of fresh water or in air, with air passing through the chamber at the rate of 20 mL min⁻¹. The rate of air-flow was controlled by a Sierra Instruments Model 90IC-PE control box and model 840-L-I-VI-S1 controller. *B. porosa* were measured in larger containers, with an air flow of either 50 or 100 mL min⁻¹, depending on their size. The O₂ concentration of the air passing into the chamber was either 1.94%, 5.0%, 12.12% or 20.94%. Mixtures of compressed O₂/N₂ were obtained from BOC Gases Australia, with the percentage O₂ certified to ±0.02%. The inflow air and water temperatures were held at a constant 20°C (±1.0). This temperature was selected as it approximated the median temperature of the water for these fish in their natural environment during summer (Pusey and Edward 1990). Fish were tested at only one O₂ concentration each day.

The rates of oxygen consumption (V_O₂: mL g⁻¹ h⁻¹) and carbon dioxide production (V_CO₂: mL g⁻¹ h⁻¹) were calculated from the composition of the excurrent air after it was passed through a Drierite column to remove water vapour. The O₂ and CO₂ contents of the excurrent dried airstream were measured with a David Bishop COMBO (model 280, galvanometric O₂ and infrared CO₂ analyser) and its voltage outputs were monitored by two Thurlby 1905A digital voltmeters and a PC via RS232 connections. V_O₂ and V_CO₂ were calculated at STPD after Withers (1977). A custom Visual Basic program was used to collect and store data. Data were analysed using a custom Excel spreadsheet. A base-line record of O₂ consumption was determined before and after the experiment, with the air mixture passing through the chamber. From the continuous recording of O₂ consumption, the metabolic data were corrected for drift (due apparently to changes in atmospheric pressure and temperature of the analyser) using the initial and final base-line values and assuming linear drift. The metabolic rate for most fish was measured over a period of 15–20 min with data collected every 10 s. An average metabolic rate was calculated typically over 3–4 min of recording after fish attained a stable metabolic recording. This was easily monitored as we continuously graphed V_O₂ and V_CO₂ during the experiment. Where possible, we also recorded the time taken for 20 opercular movements, using a stopwatch, while the metabolism of the fish was being measured in water or in air. There were no deaths during experiments.

Differences among metabolic rates for the four hypoxic conditions were tested by ANCOVA for logarithmically transformed data with body mass as the covariate. The probability for significance of statistical tests was P < 0.05.

Results

As the fish were measured over a number of days, and the same fish were not necessarily used in each experiment, the mean body mass of the three species varied slightly between experiments (Table 1). Body masses were approximately 0.48 g for *L. salamandroides*, 0.35 g for *G. nigrostriata*, and 10 g for *B. porosa*.

**Oxygen consumption**

In water, V_O₂ decreased significantly (F_{3,19} = 3.44, P < 0.05) with progressive hypoxia, from 0.29 mL g⁻¹ h⁻¹ at 21% O₂ to 0.20 mL g⁻¹ h⁻¹ at 12%, 0.11 mL g⁻¹ h⁻¹ at 5% and 2% O₂ for *L. salamandroides* (Table 1; Fig. 1). Similarly for *G. nigrostriata*, V_O₂ decreased significantly (F_{3,23} = 11.90, P < 0.05) with progressive hypoxia, from 0.48 mL g⁻¹ h⁻¹ at 21% O₂ to 0.39 mL g⁻¹ h⁻¹ at 12%, to 0.31 mL g⁻¹ h⁻¹ at 5%, to 0.08 mL g⁻¹ h⁻¹ at 2% O₂ (Fig. 2). For *B. porosa*, V_O₂ declined significantly (F_{3,23} = 12.65, P < 0.05) with progressive hypoxia, from 0.44 mL g⁻¹ h⁻¹ in 21% O₂ to 0.13 mL g⁻¹ h⁻¹ at 12%, 0.08 mL g⁻¹ h⁻¹ at 5%, and 0.02 mL g⁻¹ h⁻¹ at 2% O₂ in air (Fig. 3).
Table 1. Oxygen consumption and carbon dioxide production (mL g⁻¹ h⁻¹) for *L. salamandroides*, *G. nigrostriata* and *B. porosa* in water and in air at 20°C at various concentration of atmospheric oxygen

Values are means ± 1 s.e., with sample size (n). *P* values from ANCOVA, with mass as the covariate, are shown for difference among species or among metabolic rates, expressed as either oxygen consumption or carbon dioxide production. The mean body mass of *L. salamandroides* was approximately 0.48 g, that of *G. nigrostriata* was 0.35 g and that of *B. porosa* was 10 g.

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<th>2%</th>
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<th>21%</th>
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<tbody>
<tr>
<td><strong>L. salamandroides in water</strong></td>
<td></td>
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<tr>
<td>$\dot{V}_O_2$</td>
<td>0.11 ± 0.057 (3)</td>
<td>0.11 ± 0.067 (6)</td>
<td>0.20 ± 0.024 (8)</td>
<td>0.29 ± 0.050 (8)</td>
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<td>$\dot{V}_CO_2$</td>
<td>0.21 ± 0.026 (5)</td>
<td>0.16 ± 0.010 (8)</td>
<td>0.16 ± 0.024 (8)</td>
<td>0.29 ± 0.060 (8)</td>
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<td><strong>L. salamandroides in air</strong></td>
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<tr>
<td>$\dot{V}_O_2$</td>
<td>0.11 ± 0.055 (5)</td>
<td>0.19 ± 0.034 (3)</td>
<td>0.20 ± 0.022 (7)</td>
<td>0.18 ± 0.028 (8)</td>
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<td>$\dot{V}_CO_2$</td>
<td>0.27 ± 0.040 (8)</td>
<td>0.19 ± 0.021 (8)</td>
<td>0.18 ± 0.018 (8)</td>
<td>0.26 ± 0.029 (8)</td>
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<td>Difference in $\dot{V}_O_2$ in water and air</td>
<td>$t_5 = 0.07, P = 0.95$</td>
<td>$t_6 = 1.44, P = 0.20$</td>
<td>$t_7 = 0.08, P = 0.94$</td>
<td>$t_8 = 1.94, P = 0.08$</td>
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<td>Difference in $\dot{V}_CO_2$ in water and air</td>
<td>$t_9 = 1.35, P = 0.21$</td>
<td>$t_9 = 1.39, P = 0.20$</td>
<td>$t_9 = 0.80, P = 0.44$</td>
<td>$t_9 = 0.41, P = 0.69$</td>
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<td><strong>G. nigrostriata in water</strong></td>
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<tr>
<td>$\dot{V}_O_2$</td>
<td>0.08 ± 0.024 (5)</td>
<td>0.31 ± 0.067 (8)</td>
<td>0.39 ± 0.058 (7)</td>
<td>0.48 ± 0.083 (8)</td>
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<tr>
<td>$\dot{V}_CO_2$</td>
<td>0.11 ± 0.021 (8)</td>
<td>0.15 ± 0.023 (8)</td>
<td>0.34 ± 0.033 (8)</td>
<td>0.48 ± 0.056 (8)</td>
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<td><strong>G. nigrostriata in air</strong></td>
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<tr>
<td>$\dot{V}_O_2$</td>
<td>0.07 ± 0.011 (6)</td>
<td>0.13 ± 0.035 (8)</td>
<td>0.20 ± 0.023 (8)</td>
<td>0.21 ± 0.067 (6)</td>
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<td>$\dot{V}_CO_2$</td>
<td>0.12 ± 0.027 (8)</td>
<td>0.10 ± 0.022 (8)</td>
<td>0.15 ± 0.025 (8)</td>
<td>0.21 ± 0.019 (8)</td>
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<td>Difference in $\dot{V}_O_2$ in water and air</td>
<td>$t_5 = 0.34, P = 0.75$</td>
<td>$t_6 = 2.35, P &lt; 0.05$</td>
<td>$t_7 = 3.11, P &lt; 0.05$</td>
<td>$t_8 = 2.58, P &lt; 0.05$</td>
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<td>Difference in $\dot{V}_CO_2$ in water and air</td>
<td>$t_9 = 0.09, P = 0.93$</td>
<td>$t_9 = 1.38, P = 0.19$</td>
<td>$t_9 = 4.69, P &lt; 0.05$</td>
<td>$t_9 = 4.42, P &lt; 0.05$</td>
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<td><strong>B. porosa in water</strong></td>
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<tr>
<td>$\dot{V}_O_2$</td>
<td>0.02 ± 0.011 (6)</td>
<td>0.08 ± 0.018 (6)</td>
<td>0.13 ± 0.020 (6)</td>
<td>0.44 ± 0.057 (11)</td>
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<tr>
<td>$\dot{V}_CO_2$</td>
<td>0.11 ± 0.018 (7)</td>
<td>0.15 ± 0.016 (8)</td>
<td>0.16 ± 0.020 (6)</td>
<td>0.29 ± 0.044 (13)</td>
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<tr>
<td><strong>B. porosa in air</strong></td>
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<tr>
<td>$\dot{V}_O_2$</td>
<td>0.004 ± 0.004 (8)</td>
<td>0.01 ± 0.002 (6)</td>
<td>0.01 ± 0.009 (6)</td>
<td>0.08 ± 0.026 (10)</td>
</tr>
<tr>
<td>$\dot{V}_CO_2$</td>
<td>0.08 ± 0.011 (8)</td>
<td>0.02 ± 0.003 (6)</td>
<td>0.03 ± 0.005 (6)</td>
<td>0.08 ± 0.009 (13)</td>
</tr>
<tr>
<td>Difference in $\dot{V}_O_2$ in water and air</td>
<td>$t_5 = 1.03, P = 0.34$</td>
<td>$t_6 = 3.87, P &lt; 0.05$</td>
<td>$t_7 = 5.59, P &lt; 0.05$</td>
<td>$t_8 = 5.80, P &lt; 0.05$</td>
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<tr>
<td>Difference in $\dot{V}_CO_2$ in water and air</td>
<td>$t_9 = 1.68, P = 0.12$</td>
<td>$t_9 = 6.92, P &lt; 0.05$</td>
<td>$t_9 = 6.07, P &lt; 0.05$</td>
<td>$t_9 = 4.58, P &lt; 0.05$</td>
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</table>

Difference among species for $\dot{V}_O_2$ in water: $F_{2,11} = 3.28, P = 0.076$; $F_{2,17} = 5.97, P < 0.05$; $F_{2,18} = 11.90, P < 0.05$; $F_{2,24} = 2.31, P = 0.12$

Difference among species for $\dot{V}_CO_2$ in water: $F_{2,15} = 6.00, P < 0.05$; $F_{2,19} = 0.26, P = 0.77$; $F_{2,19} = 16.23, P < 0.05$; $F_{2,26} = 3.92, P < 0.05$

Difference among species for $\dot{V}_O_2$ in water: $F_{2,16} = 4.30, P < 0.05$; $F_{2,14} = 7.17, P < 0.05$; $F_{2,18} = 26.39, P < 0.05$; $F_{2,21} = 3.46, P < 0.05$

Difference among species for $\dot{V}_CO_2$ in water: $F_{2,21} = 13.21, P < 0.05$; $F_{2,19} = 18.41, P < 0.05$; $F_{2,19} = 13.49, P < 0.05$; $F_{2,26} = 30.75, P < 0.05$
There were no significant differences ($F_{3,17} = 1.59, P = 0.23$) in $V_O_2$ for $L. salamandroides$ in air at different $O_2$ concentrations. However, $V_O_2$ declined significantly ($F_{3,21} = 5.68, P < 0.05$) with hypoxia in air for $G. nigrostriata$ from 0.21 mL g$^{-1}$ h$^{-1}$ at 21% $O_2$ to 0.20 mL g$^{-1}$ h$^{-1}$ at 12%, to 0.13 mL g$^{-1}$ h$^{-1}$ at 5%, to 0.07 mL g$^{-1}$ h$^{-1}$ at 2% $O_2$, and even more dramatically ($F_{3,20} = 17.61, P < 0.05$) for $B. porosa$ in air, from 0.08 mL g$^{-1}$ h$^{-1}$ at 21% $O_2$, to 0.01 mL g$^{-1}$ h$^{-1}$ at 12% and 8%, to 0.004 mL g$^{-1}$ h$^{-1}$ at 2% (Table 1). Oxygen consumption was not significantly different for $L. salamandroides$ in water than in air at 21, 12 and 5% $O_2$ concentrations, but was significantly higher for $G. nigrostriata$ and $B. porosa$ in water than in air (Table 1). There was no difference between $V_O_2$ in air and water for any species at 2% $O_2$.

**Carbon dioxide production**

$V_{CO_2}$ results largely paralleled $V_O_2$ results (Figs 1–3; except as discussed below). In air and water, there was a significant difference in $V_{CO_2}$ at 21, 12, 5 and 2% $O_2$ concentrations for $G. nigrostriata$ and $B. porosa$. Although there was no significant difference in $V_{CO_2}$ at the four
O₂ concentrations for *L. salamandroides*, the values increased from 5% to 2% ambient O₂, as they did in air for *G. nigrostriata* and *B. porosa* (Table 1; Figs 1–3).

Respiratory exchange ratio (RER = V\textsubscript{CO₂}/V\textsubscript{O₂}) in all cases increased appreciably with hypoxia from 5% to 2% O₂ concentrations, with greater increases being evident in air than in water (Figs 1–3). The largest increase was for *B. porosa* in air, which reflects the very low V\textsubscript{O₂} value (0.004 mL g\(^{-1}\) h\(^{-1}\)) and the higher (compared with the values at 12 and 5%) V\textsubscript{O₂} value.

Opercular and body movements

*G. nigrostriata* were too small and the rate of gill movement too fast to accurately measure, so opercular rates were recorded only for *L. salamandroides* and *B. porosa* in water (Table 2). There was a significant difference (*F*\(_{3,28} = 3.36, P < 0.05*) among opercular rates for *L. salamandroides* at the four O₂ concentrations; a Tukey’s test indicated that the only significant difference in the rate of opercular movement for *L. salamandroides* was at an O₂ concentration of 2% compared with 12%. There was no significant difference in opercular rates for *B. porosa* among the four O₂ concentrations.

In air, *L. salamandroides* would often remain motionless in the metabolic chamber, and we could not see gulping or opercular movements, although the fish would often leave their opercular flaps open. *G. nigrostriata* would often wriggle in air, and we occasionally observed them to gasp or produce a bubble of air from their mouth, but these events were infrequent. *B.

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**Fig. 2.** Oxygen consumption (mL g\(^{-1}\) h\(^{-1}\)), carbon dioxide production and respiratory exchange ratios for *G. nigrostriata* at 21, 12, 5 and 2% O₂ concentrations in air and water. Means ± 1 s.e. are shown for metabolism. Solid lines are for in water, dotted lines are for in air at 20°C.
porosa in air would often initially wriggle and then would remain motionless with the opercular flaps remaining either open or closed; they would occasionally gulp air. Bubbles were often formed by the incoming air flow into the moist metabolic chamber containing L. salamandroides in air, and we presume that the formation of these bubbles was assisted by mucus secreted by the fish as they lay in the moist environment. Similar bubbles were not observed for G. nigrostriata or B. porosa.

Fig. 3. Oxygen consumption (mL g⁻¹ h⁻¹), carbon dioxide production and respiratory exchange ratios for B. porosa at 21, 12, 5 and 2% O₂ concentrations in air and water. Means ± 1 s.e. are shown for metabolism. Solid lines are for in water, dotted lines are for in air at 20°C.

Table 2. Rate of opercular movements for L. salamandroides and B. porosa in water at 20°C at various concentrations of atmospheric oxygen

Values are means for number of movements per minute ± 1 s.e., with sample size (n). P values are shown for difference among oxygen concentrations.

<table>
<thead>
<tr>
<th>Oxygen concentration</th>
<th>L. salamandroides</th>
<th>B. porosa</th>
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<tbody>
<tr>
<td>2%</td>
<td>114.4 ± 4.65 (8)</td>
<td>73.5 ± 3.79 (6)</td>
</tr>
<tr>
<td>5%</td>
<td>98.9 ± 4.95 (8)</td>
<td>89.7 ± 8.85 (6)</td>
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<tr>
<td>12%</td>
<td>92.8 ± 4.99 (8)</td>
<td>92.0 ± 8.52 (6)</td>
</tr>
<tr>
<td>21%</td>
<td>94.5 ± 6.69 (8)</td>
<td>89.9 ± 5.06 (8)</td>
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\( F_{3,28} = 3.36, \ P < 0.05 \)

\( F_{3,22} = 1.55, \ P = 0.23 \)
Discussion

Few physiological data are available for any aestivating fish (Smith 1930, 1935; Rosa 1977; Bicudo and Johansen 1979; Pusey 1989b), particularly small species. Aestivating fish face a number of major physiological problems, including gas exchange, water balance, accumulation or excretion of metabolic wastes, and economy of energy (Pusey 1989a). Fishes that burrow into the substrate and remain above the water table must be capable of respiring in a saturated air environment. *L. salamandroides* and *G. nigrostriata* have adapted to these potential stresses and are able to survive the absence of surface water (Berra and Allen 1989; Allen and Berra 1989; Pusey 1990), whereas the sympatric *B. porosa* has not been reported to aestivate. We interpret the metabolic physiology of these fishes in air, and with hypoxia, as supporting evidence for aestivation in moist soil or in remaining water pockets in the soil, or for an inability to aestivate.

*L. salamandroides*

The metabolic pattern that we measured for *L. salamandroides* in air and water, and with hypoxia, differed from that of the other two species: there was a low VO$_2$ that was little affected in air (0.29 mL g$^{-1}$ h$^{-1}$) or with hypoxia (0.18 mL g$^{-1}$ h$^{-1}$). Pusey (1986) reported a much lower metabolic rate for 0.88-g *L. salamandroides* at 20°C in water (0.05 mL g$^{-1}$ h$^{-1}$) and Pusey (1989a) reported 0.14 mL g$^{-1}$ h$^{-1}$ in air. Martin et al. (1993) subsequently calculated a similar value in air to ours at 22°C of 0.25 mL g$^{-1}$ h$^{-1}$ (from their fig. 1). Our metabolic data for *L. salamandroides* suggests adaptations to both aerial exposure and hypoxia.

Berra et al. (1989) reported that *L. salamandroides* is unlikely to use its swim bladder as a respiratory organ and suggested that it survives aestivation by one of four strategies: (a) it burrows in the moist substrate and tightly closes its gill chambers with its opercular flaps, trapping moisture around the gills, and reduces its metabolic rate, (b) it switches to anaerobic respiration, (c) it utilises cutaneous respiration in a moist environment, or (d) it moves into underground sources of water where it utilises its gill or skin for gas exchange. *L. salamandroides*, unlike *G. nigrostriata* and *B. porosa*, shows no significant differences between VO$_2$ (and VCO$_2$) in water and air at any O$_2$ concentration, suggesting that it adopts a different strategy to survive the lack of surface water. *L. salamandroides* probably does not adopt strategies (a) or (b) of Berra et al. (1989) as it will open its operculum and has a substantial VO$_2$ in air, but it could adopt either of options (c) or (d) of Berra et al. (1989). Berra and Allen (1989) provide evidence for option (c) as they reported digging up *L. salamandroides* from moist substrate at the top of the ground water table, 4–9 cm below the stream bed and 20 cm from the edge of the pool. Allen and Berra (1989) provide evidence for option (d) as they found a single *L. salamandroides* in water at the bottom of a freshwater crayfish burrow and not in a state of ‘torpor’, as described by Pusey (1981). *L. salamandroides* can obtain a high proportion of its O$_2$ requirement via its skin in water (Martin et al. 1993); this, together with its low metabolic rate (Pusey 1989a, this study), no gasping, no opercular movements or other visible respiratory behaviour (Martin et al. 1993; our data), supports options (c) and (d). The comparatively high skin-surface-area-to-body-mass ratio for these small fish compared with, for example, lungfish, may mean that they can survive out of water by reducing their metabolism and relying on cutaneous respiration (option c) as long as their skin remains moist, a view supported by Martin et al. (1993).

If *L. salamandroides* survives in a burrow or small chamber in the substrate with or without a surrounding medium of water, it is likely that pO$_2$ will decline below that on the surface. In addition, the ephemeral swamps and ponds with substantial quantities of decaying organic matter can be hypoxic, particularly in the leaf litter at the bottom where *L. salamandroides* spend most of their time (Pusey and Edward 1990). *L. salamandroides* does not appear to be stressed or reduce its metabolic rate to live in these conditions in a laboratory situation until hypoxia reaches 2% O$_2$. At O$_2$ concentrations of 21, 12 and 5%, *L. salamandroides* seems to cope equally well metabolically in air and water. However, under very hypoxic conditions of 2% O$_2$, VO$_2$ might decrease slightly but VCO$_2$ increases relative to O$_2$ consumption, particularly in
air. This is reflected in the high RER values at 2% O2 (Fig. 1) and suggests that CO2 is being released from the body fluids at a greater rate than VO2, is used. One explanation for this is that the fish may switch to anaerobic metabolism at 2% O2, and a consequently lowered pH releases CO2 from the tissues. Survival at 2% O2 may therefore be temporary. A similar or even more pronounced effect of hypoxia on VO2, VCO2 and RER is apparent for G. nigrostriata and B. porosa.

L. salamandroides forms a thick layer of mucus around its body during aestivation, presumably to minimise water loss (Pusey 1986). In our respirometry experiments, air bubbled through the chamber containing L. salamandroides often formed small bubbles, presumably assisted by the mucus coating that rubbed off the salamanderfish onto the inside of the chamber; this did not occur for G. nigrostriata or B. porosa.

Galaxiella nigrostriata

The black-striped minnow has also been found in the substrate when the ponds have dried up (Berra and Allen 1989). In contrast to that of L. salamandroides, the metabolic rate of G. nigrostriata was significantly higher in water than in air at O2 concentrations of 21, 12 and 5%, although at 2% O2 the rates were almost equally low (and similar to those of L. salamandroides: Fig. 4). With progressive hypoxia, VO2 of G. nigrostriata in water decreased significantly from 5% to 2% O2 (t10 = 3.25) whereas VCO2 declined significantly from 12% to 5% (Fig. 2). In air, VCO2 increases slightly from an O2 concentration of 5% to 2% while VO2 continues to decline. This suggests that G. nigrostriata is coping metabolically with the lower concentrations of O2 at 5% but at 2% there is probably insufficient O2, and survival of G. nigrostriata may be limited to short periods of hypoxia.

For G. nigrostriata, the metabolic rate declines as the concentration of O2 decreases, unlike the situation for L. salamandroides. G. nigrostriata does not appear to secrete mucus in air, and its movements on soil are much less ‘effective’ than L. salamandroides, and its burrowing into the substrate was observed to be less proficient than that of L. salamandroides in a laboratory situation. These observations suggest that its survival strategy during the period when the ponds dry up is different to that of L. salamandroides. G. nigrostriata may adopt option (d) of Berra et al. (1989), moving into the underground water sources, possibly in the chimneys created by the freshwater crayfish (Cherax preissii) to survive the period of no surface water.

We noticed small rapid increases in VO2 for G. nigrostriata in air that appeared to coincide with head movements that may have been gulps of air. All six G. nigrostriata measured in air at 21% O2 showed such pulses in VO2, but these were not evident at 12, 5 or 2% O2 (nor were they evident for L. salamandroides or B. porosa at any O2 concentration).

Bostockia porosa

B. porosa is larger than L. salamandroides and G. nigrostriata, with males and females growing to a total length of 105 mm in their sixth year (Pen and Potter 1990). In water, at 21% O2 concentration, there were no significant differences among the three species in metabolic rate although the mass-specific metabolic rate of B. porosa would be expected to be lower because of the allometric effect of its larger mass. In air, the metabolic rate of B. porosa is much lower than that of L. salamandroides at an O2 concentration of 12, 5 and 2% and lower than that of G. nigrostriata at 5% O2 concentration. These data suggest that B. porosa is unable to maintain effective gas exchange in air (like, for example, goldfish: Hillman and Withers 1987).

Both VO2 and VCO2 decline significantly for B. porosa with hypoxia, in both air and water. VO2 for B. porosa at 12% and 2% O2 concentrations in air is not significantly different from zero, and the value at 5% O2 concentration (0.01 mL g−1 h−1) is very close to zero. The respirometry recordings for B. porosa indicated pulses in O2 consumption that we interpreted as gas released from the swim bladder having a different pO2 than ambient air. There was no significant increase in the rate of opercular movements in water with hypoxia, suggesting no attempt to increase ventilation or oxyconform. VCO2 is significantly higher than VO2 in water and
Fig. 4. Oxygen consumption in air and in water for *L. salamandroides*, *G. nigrostriata* and *B. porosa*. Solid lines are for VO$_2$ in water and dotted lines for VO$_2$ in air at 20°C.
air at 2% O_2 (t_0 = 2.68 and 4.48, respectively) and the very high RER (~16) at 2% ambient O_2 concentration in air reflects a higher CO_2 release (than at 12% and 5% O_2) even though the V_O_2 is very low. This might suggest that B. porosa is using anaerobic metabolic pathways that reduce body fluid pH and consequentially release CO_2. B. porosa is therefore unlikely to survive at 2% O_2 for an extended period.

**Summary**

There is no clear general pattern for the V_O_2 of fishes in water compared with in air (Graham 1997). However, V_O_2 is generally higher in air than in water for active amphibious fishes and V_O_2 is generally lower in air than in water for normal aquatic species that remain quiescent during temporary exposure to air. For B. porosa, which seems to be a typically aquatic fish, metabolism in water is much higher than in air, consistent with Graham’s (1997) suggestion. For the more benthic and terrestrially mobile L. salamandroides, which also aestivates in moist soil, there was no significant difference between V_O_2 in air and water, presumably reflecting its normally low metabolic rate and capacity to exchange gases cutaneously in air. For G. nigrostriata, V_O_2 was reduced in air, but not as much as for B. porosa, suggesting some limited capacity for cutaneous gas exchange.

Progressive hypoxia decreased the metabolic rate for all three fishes. B. porosa has a ‘typical’ oxyconforming pattern for V_O_2 in water, whereas L. salamandroides does not and G. nigrostriata is somewhat intermediate (Fig. 4). We attribute these differences to the activity pattern of these fishes. The more sedentary and benthic L. salamandroides in water moves little compared with G. nigrostriata and B. porosa. G. nigrostriata swims in mid-water, and is sometimes seen in schools whereas B. porosa is a mid-to-bottom water fish that is active at night. The metabolism of G. nigrostriata declines in air as hypoxia changes from 12% to 2% whereas a decline is evident for L. salamandroides only at 2% O_2 (Fig. 4). B. porosa does not appear to cope out of water and therefore would not be able to aestivate even in a saturated environment.

**Acknowledgments**

This project was funded by a small ARC grant administered by Edith Cowan University. Experimentation was undertaken with the approval of the Animal Ethics Committee of the University of Western Australia. Fish were caught under licence from the Department of Conservation and Land Management.

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Manuscript received 28 January 1999; accepted 27 May 1999

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