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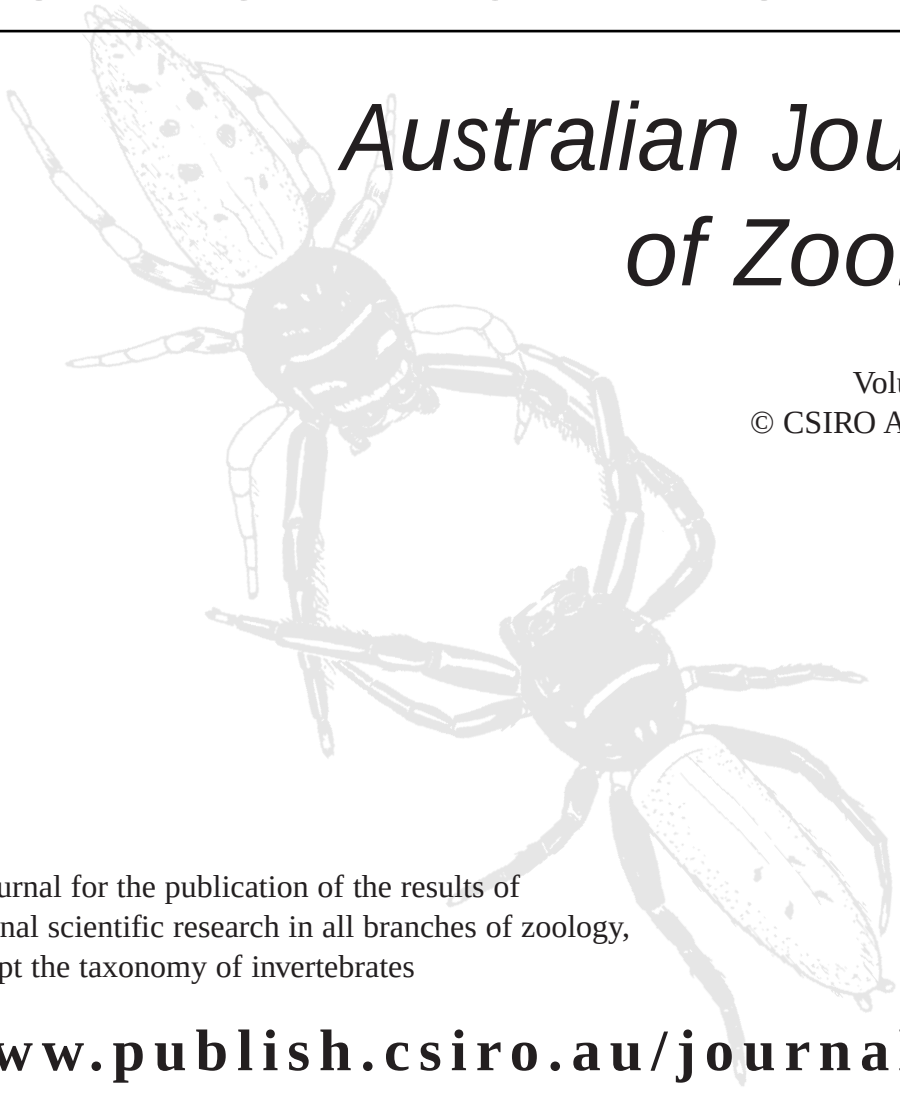

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The metabolic response to hypoxia and emersion of aestivating fishes (*Lepidogalaxias salamandroides* and *Galaxiella nigrostriata*) and a non-aestivating fish (*Bostockia porosa*) from south-western Australia

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Abstract

We measured the metabolic rate of three fishes (*Lepidogalaxias salamandroides,* G*alaxiella 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 $\dot{V}_{O_2}$ in water (0.29 mL g^{-1} h^{-1}) and air (0.18 mL g^{-1} h^{-1}). Progressive hypoxia (12, 5 and 2% O_2) 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% O_2). *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 O_2 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 1989*b*; 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 1989*b*; 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

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hypoxic conditions (12, 5 and 2% O_2 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 O_2 concentration of the air passing into the chamber was either 1.94%, 5.0%, 12.12% or 20.94%. Mixtures of compressed O_2/N_2 were obtained from BOC Gases Australia, with the percentage O_2 certified to ±0.02%. The inflow air and water temperatures were held at a constant 20ºC (±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 O_2 concentration each day.

The rates of oxygen consumption ($\dot{V}_{O_2}$: mL g^{-1} h^{-1}) and carbon dioxide production ($\dot{V}_{CO_2}$: 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 O_2 and CO_2 contents of the excurrent dried airstream were measured with a David Bishop COMBO (model 280, galvanometric O_2 and infrared CO_2 analyser) and its voltage outputs were monitored by two Thurlby 1905A digital voltmeters and a PC via RS232 connections. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ 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 O_2 consumption was determined before and after the experiment, with the air mixture passing through the chamber. From the continuous recording of O_2 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 $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ 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, $\dot{V}_{O_2}$ decreased significantly ($F_{3,19} = 3.44$, $P < 0.05$) with progressive hypoxia, from 0.29 mL g^{-1} h^{-1} at 21% O_2, to 0.20 mL g^{-1} h^{-1} at 12%, 0.11 mL g^{-1} h^{-1} at 5% and 2% O_2 for *L. salamandroides* (Table 1; Fig. 1). Similarly for *G. nigrostriata*, $\dot{V}_{O_2}$ decreased significantly ($F_{3,23} = 11.90$, $P < 0.05$) with progressive hypoxia, from 0.48 mL g^{-1} h^{-1} at 21% O_2 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% O_2 (Fig. 2). For *B. porosa,* $\dot{V}_{O_2}$ declined significantly ($F_{3,23} = 12.65$, $P < 0.05$) with progressive hypoxia, from 0.44 mL g^{-1} h^{-1} in 21% O_2 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% O_2 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

	Oxygen concentration				
	2%	5%	12%	21%	
L. salamandroides in water					
$\dot{V}_{O_2}$	0.11 ± 0.057 (3)	0.11 ± 0.067 (6)	0.20 ± 0.024 (8)	0.29 ± 0.050 (8)	$F_{3,19} = 3.44$, $P < 0.05$
$\dot{V}_{CO_2}$	0.21 ± 0.026 (5)	0.16 ± 0.010 (8)	0.16 ± 0.024 (8)	0.29 ± 0.60 (8)	$F_{3,24} = 1.27$, $P = 0.31$
L. salamandroides in air					
$\dot{V}_{O_2}$	0.11 ± 0.055 (5)	0.19 ± 0.034 (3)	0.20 ± 0.022 (7)	0.18 ± 0.028 (8)	$F_{3,17} = 1.59$, $P = 0.23$
$\dot{V}_{CO_2}$	0.27 ± 0.040 (8)	0.19 ± 0.021 (8)	0.18 ± 0.018 (8)	0.26 ± 0.029 (8)	$F_{3,27} = 2.14$, $P = 0.12$
Difference in $\dot{V}_{O_2}$ in water and air	$t_5 = 0.07$, $P = 0.95$	$t_6 = 1.44$, $P = 0.20$	$t_{12} = 0.08$, $P = 0.94$	$t_{10} = 1.94$, $P = 0.08$	
Difference in $\dot{V}_{CO_2}$ in water and air	$t_{10} = 1.35$, $P = 0.21$	$t_9 = 1.39$, $P = 0.20$	$t_{13} = 0.80$, $P = 0.44$	$t_{10} = 0.41$, $P = 0.69$	
G. nigrostriata in water					
$\dot{V}_{O_2}$	0.08 ± 0.024 (5)	0.31 ± 0.067 (8)	0.39 ± 0.058 (7)	0.48 ± 0.083 (8)	$F_{3,23} = 11.90$, $P < 0.05$
$\dot{V}_{CO_2}$	0.11 ± 0.021 (8)	0.15 ± 0.023 (8)	0.34 ± 0.033 (8)	0.48 ± 0.056 (8)	$F_{3,27} = 18.22$, $P < 0.05$
G. nigrostriata in air					
$\dot{V}_{O_2}$	0.07 ± 0.011 (6)	0.13 ± 0.035 (8)	0.20 ± 0.023 (8)	0.21 ± 0.067 (6)	$F_{3,21} = 5.68$, $P < 0.05$
$\dot{V}_{CO_2}$	0.12 ± 0.027 (8)	0.10 ± 0.022 (8)	0.15 ± 0.025 (8)	0.21 ± 0.019 (8)	$F_{3,27} = 3.16$, $P < 0.05$
Difference in $\dot{V}_{O_2}$ in water and air	$t_5 = 0.34$, $P = 0.75$	$t_{10} = 2.35$, $P < 0.05$	$t_7 = 3.11$, $P < 0.05$	$t_{11} = 2.58$, $P < 0.05$	
Difference in $\dot{V}_{CO_2}$ in water and air	$t_{13} = 0.09$, $P = 0.93$	$t_{13} = 1.38$, $P = 0.19$	$t_{13} = 4.69$, $P < 0.05$	$t_8 = 4.42$, $P < 0.05$	
B. porosa in water					
$\dot{V}_{O_2}$	0.02 ± 0.011 (6)	0.08 ± 0.018 (6)	0.13 ± 0.020 (6)	0.44 ± 0.057 (11)	$F_{3,23} = 12.65$, $P < 0.05$
$\dot{V}_{CO_2}$	0.11 ± 0.018 (7)	0.15 ± 0.018 (6)	0.16 ± 0.020 (6)	0.29 ± 0.044 (13)	$F_{3,26} = 5.84$, $P < 0.05$
B. porosa in air					
$\dot{V}_{O_2}$	0.004 ± 0.004 (8)	0.01 ± 0.002 (6)	0.01 ± 0.009 (6)	0.08 ± 0.026 (10)	$F_{3,20} = 17.61$, $P < 0.05$
$\dot{V}_{CO_2}$	0.08 ± 0.011 (8)	0.02 ± 0.003 (6)	0.03 ± 0.005 (6)	0.08 ± 0.009 (13)	$F_{3,28} = 5.41$, $P < 0.05$
Difference in $\dot{V}_{O_2}$ in water and air	$t_6 = 1.03$, $P = 0.34$	$t_5 = 3.87$, $P < 0.05$	$t_7 = 5.59$, $P < 0.05$	$t_{13} = 5.80$, $P < 0.05$	
Difference in $\dot{V}_{CO_2}$ in water and air	$t_{10} = 1.68$, $P = 0.12$	$t_5 = 6.92$, $P < 0.05$	$t_5 = 6.07$, $P < 0.05$	$t_{12} = 4.58$, $P < 0.05$	
Difference among species for $\dot{V}_{O_2}$ in water	$F_{2,11} = 3.28$, $P = 0.76$	$F_{2,17} = 5.97$, $P < 0.05$	$F_{2,18} = 11.90$, $P < 0.05$	$F_{2,24} = 2.31$, $P = 0.12$	
Difference among species for $\dot{V}_{CO_2}$ in water	$F_{2,17} = 6.00$, $P < 0.05$	$F_{2,19} = 0.26$, $P = 0.77$	$F_{2,19} = 16.23$, $P < 0.05$	$F_{2,26} = 3.92$, $P < 0.05$	
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	$F_{2,21} = 13.21$, $P < 0.05$	$F_{2,19} = 18.41$, $P < 0.05$	$F_{2,19} = 13.49$, $P < 0.05$	$F_{2,26} = 30.75$, $P < 0.05$	

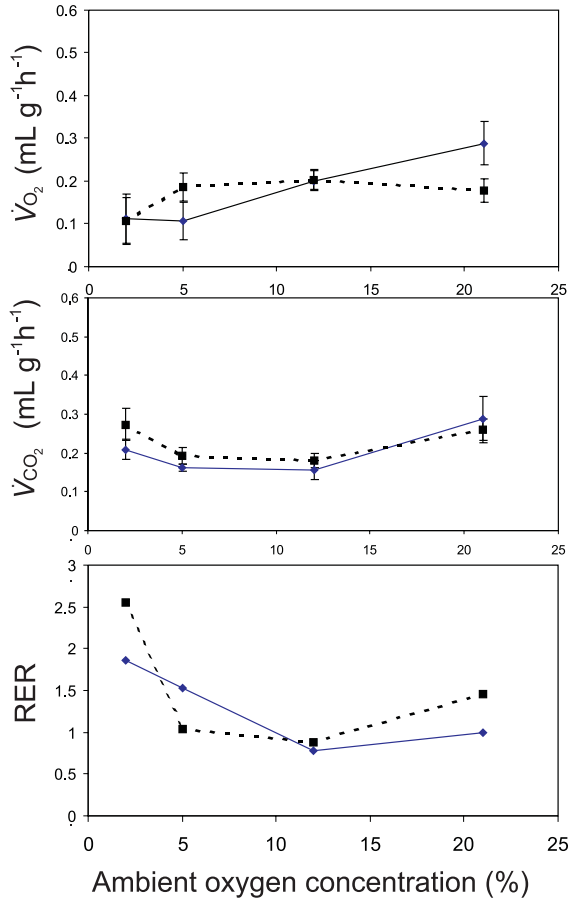

Fig. 1. Oxygen consumption (mL g^{-1} h^{-1}), carbon dioxide production and respiratory exchange ratios for *L. salamandroides* at 21, 12, 5 and 2% oxygen concentrations in air and water. Means ± 1 s.e. are shown for metabolism. Solid lines are for values in water, dotted lines are for values in air at 20ºC.

There were no significant differences ($F_{3,17}$ = 1.59, P = 0.23) in $\dot{V}_{O_2}$ for *L. salamandroides* in air at different O_2 concentrations. However, $\dot{V}_{O_2}$ 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 $\dot{V}_{O_2}$ in air and water for any species at 2% O_2 .

Carbon dioxide production

$\dot{V}_{CO_2}$ results largely paralleled $\dot{V}_{O_2}$ results (Figs 1–3; except as discussed below). In air and water, there was a significant difference in $\dot{V}_{CO_2}$ at 21, 12, 5 and 2% O_2 concentrations for *G. nigrostriata* and *B. porosa*. Although there was no significant difference in $\dot{V}_{CO_2}$ at the four

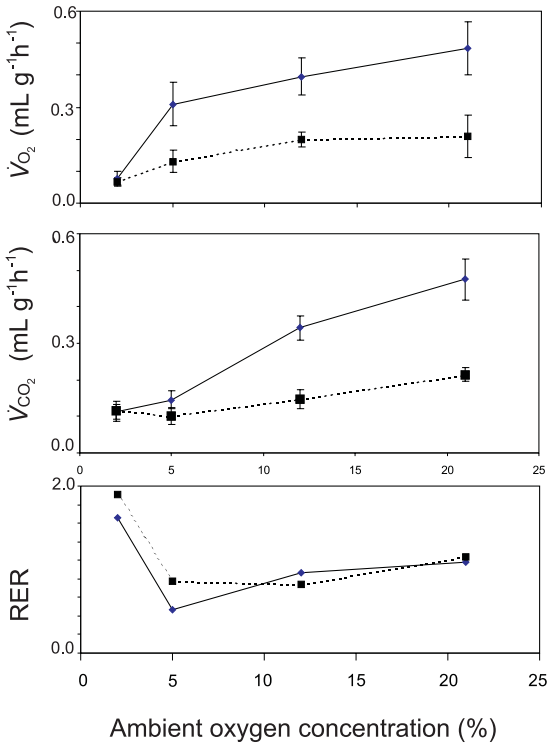

Fig. 2. Oxygen consumption (mL g^{-1} h^{-1}), carbon dioxide production and respiratory exchange ratios for *G. nigrostriata* at 21, 12, 5 and 2% O_2 concentrations in air and water. Means ± 1 s.e. are shown for metabolism. Solid lines are for in water, dotted lines are for in air at 20ºC.

O_2 concentrations for *L. salamandroides*, the values increased from 5% to 2% ambient O_2, as they did in air for *G. nigrostriata* and *B. porosa* (Table 1; Figs 1–3).

Respiratory exchange ratio (RER = $\dot{V}_{CO_2}/\dot{V}_{O_2}$) in all cases increased appreciably with hypoxia from 5% to 2% O_2 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 $\dot{V}_{O_2}$ value (0.004 mL g^{-1} h^{-1}) and the higher (compared with the values at 12 and 5%) $\dot{V}_{O_2}$ 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 O_2 concentrations; a Tukey's test indicated that the only significant difference in the rate of opercular movement for *L. salamandroides* was at an O_2 concentration of 2% compared with 12%. There was no significant difference in opercular rates for *B. porosa* among the four O_2 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.*

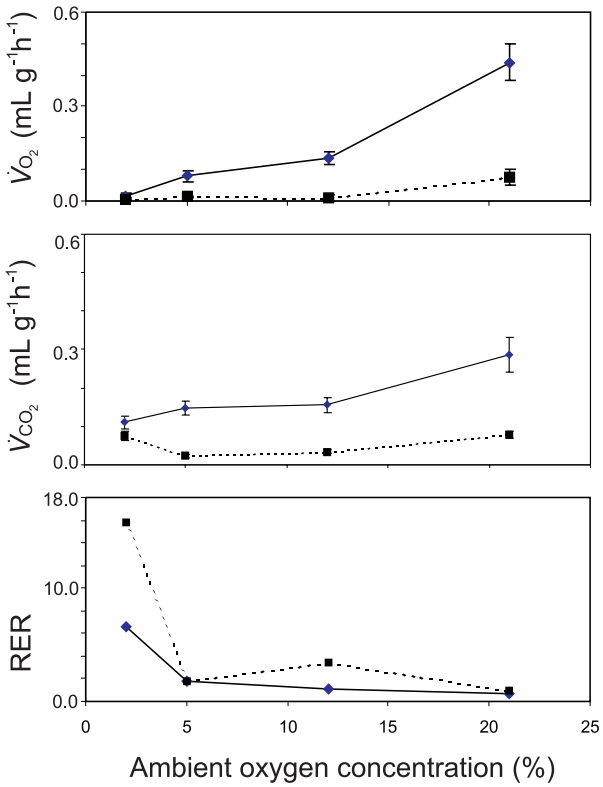

Fig. 3. Oxygen consumption (mL g^{-1} h^{-1}), carbon dioxide production and respiratory exchange ratios for *B. porosa* at 21, 12, 5 and 2% O_2 concentrations in air and water. Means ± 1 s.e. are shown for metabolism. Solid lines are for in water, dotted lines are for in air at 20ºC.

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

Oxygen concentration	*L. salamandroides*	*B. porosa*
2%	114.4 ± 4.65 (8)	73.5 ± 3.79 (6)
5%	98.9 ± 4.95 (8)	89.7 ± 8.85 (6)
12%	92.8 ± 4.99 (8)	92.0 ± 8.52 (6)
21%	94.5 ± 6.69 (8)	89.9 ± 5.06 (8)
	$F_{3,28} = 3.36$, $P < 0.05$	$F_{3,22} = 1.55$, $P = 0.23$

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*.

Discussion

Few physiological data are available for any aestivating fish (Smith 1930, 1935; Rosa 1977; Bicudo and Johansen 1979; Pusey 1989*b*), 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 1989*a*). 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.

Lepidogalaxias 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 $\dot{V}_{O_2}$ 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 20ºC in water (0.05 mL g^{-1} h^{-1}) and Pusey (1989*a*) reported 0.14 mL g^{-1} h^{-1} in air. Martin *et al.* (1993) subsequently calculated a similar value in air to ours at 22ºC 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 $\dot{V}_{O_2}$ (and $\dot{V}_{CO_2}$) 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 $\dot{V}_{O_2}$ 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 1989*b*, 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, $\dot{V}_{O_2}$ might decrease slightly but $\dot{V}_{CO_2}$ increases relative to O_2 consumption, particularly in

air. This is reflected in the high RER values at 2% O_2 (Fig. 1) and suggests that CO_2 is being released from the body fluids at a greater rate than $\dot{V}_{O_2}$ is used. One explanation for this is that the fish may switch to anaerobic metabolism at 2% O_2, and a consequently lowered pH releases CO_2 from the tissues. Survival at 2% O_2 may therefore be temporary. A similar or even more pronounced effect of hypoxia on $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ 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 O_2 concentrations of 21, 12 and 5%, although at 2% O_2 the rates were almost equally low (and similar to those of *L. salamandroides*: Fig. 4). With progressive hypoxia, $\dot{V}_{O_2}$ of *G. nigrostriata* in water decreased significantly from 5% to 2% O_2 ($t_{10} = 3.25$) whereas $\dot{V}_{CO_2}$ declined significantly from 12% to 5% (Fig. 2). In air, $\dot{V}_{CO_2}$ increases slightly from an O_2 concentration of 5% to 2% while $\dot{V}_{O_2}$ continues to decline. This suggests that *G. nigrostriata* is coping metabolically with the lower concentrations of O_2 at 5% but at 2% there is probably insufficient O_2, and survival of *G. nigrostriata* may be limited to short periods of hypoxia.

For *G. nigrostriata*, the metabolic rate declines as the concentration of O_2 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 $\dot{V}_{O_2}$ 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% O_2 showed such pulses in $\dot{V}_{O_2}$, but these were not evident at 12, 5 or 2% O_2 (nor were they evident for *L. salamandroides* or *B. porosa* at any O_2 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% O_2 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 O_2 concentration of 12, 5 and 2% and lower than that of *G. nigrostriata* at 5% O_2 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 $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ decline significantly for *B. porosa* with hypoxia, in both air and water. $\dot{V}_{O_2}$ for *B. porosa* at 12% and 2% O_2 concentrations in air is not significantly different from zero, and the value at 5% O_2 concentration (0.01 mL g^{-1} h^{-1}) is very close to zero. The respirometry recordings for *B. porosa* indicated pulses in O_2 consumption that we interpreted as gas released from the swim bladder having a different pO_2 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. $\dot{V}_{CO_2}$ is significantly higher than $\dot{V}_{O_2}$ in water and

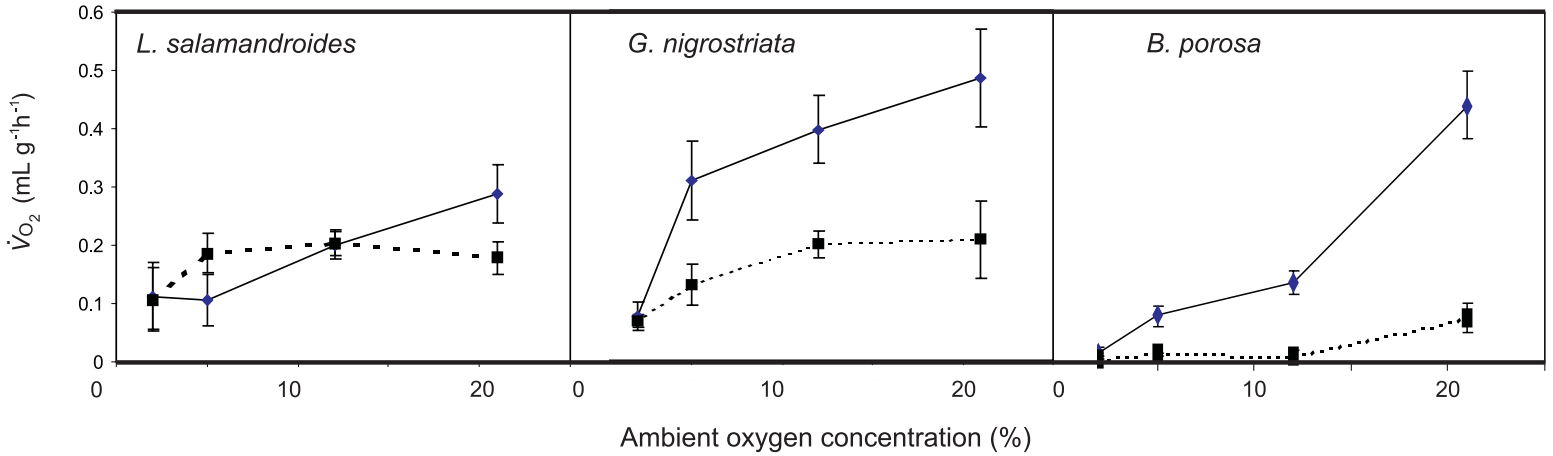

Fig. 4. Oxygen consumption in air and in water for *L. salamandroides*, *G. nigrostriata* and *B. porosa*. Solid lines are for VO_2 in water and dotted lines for VO_2 in air at 20°C.

air at 2% O_2 (t_9 = 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 $\dot{V}_{O_2}$ 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 $\dot{V}_{O_2}$ of fishes in water compared with in air (Graham 1997). However, $\dot{V}_{O_2}$ is generally higher in air than in water for active amphibious fishes and $\dot{V}_{O_2}$ 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 $\dot{V}_{O_2}$ in air and water, presumably reflecting its normally low metabolic rate and capacity to exchange gases cutaneously in air. For *G. nigrostriata*, $\dot{V}_{O_2}$ 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 $\dot{V}_{O_2}$ 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.

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