Environmental modulation of metabolic allometry in ornate rainbowfish *Rhadinocentrus ornatus*

H. Fabian Vaca and Craig R. White*

School of Biological Sciences, The University of Queensland,
St Lucia, Queensland 4072, Australia

*Author for correspondence (craig.white@uq.edu.au).

The nature of the relationship between the metabolic rate (MR) and body mass (M) of animals has been the source of controversy for over seven decades, with much of the focus on the value of the scaling exponent b , where MR is proportional to M^b . While it is well known that MR does not generally scale isometrically (i.e. b is seldom equal to 1), the value of b remains the subject of heated debate. In the present study, we examine the influence of an ecologically relevant abiotic variable, pH, on the metabolic allometry of an Australian freshwater fish, *Rhadinocentrus ornatus*. We show that the value of b is lower for rainbowfish acclimated to acidic (pH 5.0) conditions compared to rainbowfish acclimated to alkaline conditions (pH 8.5), but that acute exposure to altered pH does not alter the value of b . This significant effect of an abiotic variable on metabolic allometry supports a growing body of evidence that there is no universal value of b and demonstrates that experimental manipulations of metabolic allometry represent powerful, and as yet underused, tools to understand the factors that constrain and influence the allometry of metabolic rate.

Keywords: scaling; basal metabolite rate; quarter-power

1. INTRODUCTION

The relationship between metabolic rate and body mass is commonly described by the allometric equation $MR = aM^b$, where MR is metabolic rate, a is a normalization constant depending on the species and metabolic level, M the body mass and b is the metabolic scaling exponent, which is usually, but not always, calculated as the slope of the relationship between $\log MR$ and $\log M$ (Glazier 2005). The influence of body mass on the MR of organisms has been the subject of debate since Kleiber (1932) reported an exponent of $3/4$, which remains in widespread use (e.g. Moses *et al.* 2008; Savage *et al.* 2008; Allen & Gillooly 2009; Morlon *et al.* 2009) despite lacking universal acceptance (Dodds *et al.* 2001; Glazier 2005; White *et al.* 2007). For example, intraspecific scaling exponents change during ontogeny (Killen *et al.* 2007; Frappell 2008) and differ from interspecific ones (Glazier 2005), while interspecific exponents

differ between ectotherms and endotherms, and vary with metabolic intensity (White *et al.* 2007; Glazier 2008).

While interspecific and ontogenetic studies are relatively common, manipulative examinations of the effect of environmental parameters on the allometry of MR remain scarce (see Jeyasingh 2007; Ohlberger *et al.* 2007 for two recent examples). Such studies have the potential to be valuable because they may provide an opportunity to examine experimentally the mechanistic basis of variation in the allometry of MR. For example, some studies have demonstrated an effect of pH on MR (e.g. Hargis 1975; Ultsch *et al.* 1980), which arises as a consequence of the effects of pH on osmoregulation, respiration and O_2 and CO_2 transport. Low pH results in a loss of body electrolytes, particularly Na^+ and Cl^- (Packer & Dunson 1970; Leivestad & Muniz 1976), reductions in the rates of Na^+ and Cl^- uptake (McWilliams 1980), increased rates of gill ventilation associated with reduced blood pH and O_2 carrying capacity (Packer & Dunson 1970; Hargis 1975), and coagulation of mucus covering the gill filaments that increases the diffusion distances for O_2 and CO_2 and probably increases CO_2 retention, leading to further decreases in blood pH (Westfall 1945; Ultsch & Gros 1979).

An effect of pH on the scaling of MR might arise because, when compared to large fishes, small fishes have higher mass-specific rates of metabolism (e.g. Killen *et al.* 2007) and are likely to have blood with lower O_2 affinity (Schmidt-Nielsen & Larimer 1958) and greater pH sensitivity (i.e. a greater Bohr shift: Riggs 1960). Thus, the problems associated with life at a low pH are likely to be size-dependent, and we hypothesize that this will manifest as a difference in the allometry of MR between individuals exposed to low and high pH.

2. MATERIAL AND METHODS

The ornate rainbowfish *Rhadinocentrus ornatus* (Melanotaeniidae, Atheriniformes) is a freshwater fish found in coastal lowland heath and rainforest ecosystems of eastern Australia, where they inhabit naturally acidic waters and have been recorded at pH values as low as 4.3 (Bayly *et al.* 1975). Rainbowfish were maintained on a diet of *Artemia*, which were provided every second day.

One hundred and twenty fish were randomly assigned to four replicate 60 l tanks and were acclimated to either acid (pH 5.0, $n = 30$ per tank) or alkaline (pH 8.5, $n = 30$ per tank) conditions for a period of 28–35 days (temperature = $23.5 \pm 1.0^\circ C$; dissolved $O_2 = 6.5 \pm 0.5 \text{ mg l}^{-1}$).

Following acclimation, resting MR was measured using closed system respirometry. Fish were held within an aerated 390 ml glass chamber and fasted for 48 h prior to measurement. The chamber was then sealed, and O_2 saturation of water was measured using a fibre-optic oxygen sensor (Ocean Optics FOXY-R, Lastek, Adelaide, Australia) connected to a temperature-compensated O_2 meter (TauTheta MFPP-100-2, Lastek). The decline in O_2 saturation was continuously measured for a period of 70 min; data obtained during the first 10 min were discarded. Fish were then weighed to 0.001 g, and MR was calculated as the rate of O_2 consumption following Alton *et al.* (2007).

Fish were then maintained under acclimation conditions for a further 28 days, but were not uniquely identified. Acid-acclimated individuals were then exposed for 48 h to aerated water at pH 8.5, and alkaline individuals were exposed for 48 h to aerated water at pH 5.0. MR and body mass were then measured as described above.

Log-transformed data were analysed using STATISTIXL v. 1.8 (www.statistixl.com) and JMP v. 7.0.2 (SAS Institute, NC, USA). Differences between acclimation groups and between acute exposure groups were examined by testing the significance of a group $\times$ $\log M$ interaction, with group and $\log M$ as fixed factors,

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2009.0610> or via <http://rsbl.royalsocietypublishing.org>.

Figure 1. The effect of acclimation pH (pH_{acc}) and measurement pH (pH_{meas}) on the allometry of the MR in rainbowfish. Solid lines are the significant relationships between mass (g) and MR ($\text{ml O}_2 \text{ h}^{-1}$); dashed lines are 95 per cent confidence limits; b is the allometric scaling exponent $\pm$ s.e. (a) pH_{acc} 5.0, pH_{meas} 5.0, $b = 0.42 \pm 0.09$; (b) pH_{acc} 8.5, pH_{meas} 8.5, $b = 0.78 \pm 0.12$; (c) pH_{acc} 5.0, pH_{meas} 8.5, $b = 0.39 \pm 0.09$; (d) pH_{acc} 8.5, pH_{meas} 5.0, $b = 0.67 \pm 0.11$.

and tank (nested within group) as a random factor. To ensure that the relationship between $\log M$ and $\log \text{MR}$ was linear and that the analyses were not unduly influenced by outliers and high leverage points, the analysis was then repeated with (i) a quadratic term included to check for nonlinearity in the relationship between $\log M$ and $\log \text{MR}$ and (ii) with data excluded for the largest fish acclimated to pH 5.0, the two largest fish acclimated to pH 8.5, and any value with a studentized residual greater than 2. Scaling exponents are presented $\pm$ s.e., unless otherwise indicated, and α was set at 0.05 for all tests.

3. RESULTS AND DISCUSSION

The intraspecific scaling exponent of MR differed significantly between rainbowfish acclimated to acid and alkaline conditions, with the scaling exponent being higher under alkaline conditions ($F_{1,70} = 6.6$, $p = 0.01$; figure 1a,b). Similarly, the scaling exponent differed between fish acutely exposed to acid and alkaline conditions following acclimation to alkaline and acid conditions, respectively ($F_{1,55} = 6.2$, $p = 0.02$; figure 1b,c). Again, the scaling exponent was higher for animals acclimated to alkaline conditions. Quadratic terms were not significant in any case, indicating that the relationship between $\log M$ and $\log \text{MR}$ is linear (p -values for quadratic terms: figure 1a, $p = 0.88$; figure 1b, $p = 0.17$; figure 1c, $p = 0.27$; figure 1d, $p = 0.43$). Interaction terms remained significant for both the acclimation and acute comparisons when outliers and high leverage points were excluded ($p = 0.03$ and 0.04 , respectively, see electronic supplementary material for details). Thus, we conclude that, in ornate rainbowfish *R. ornatus*, the allometry of MR is modulated in response to environmental pH (figure 1, table 1).

While it is clear that pH alters the allometry of MR, the results of the present study do not reveal the causes

Table 1. Allometric equations for rainbowfish acclimated and measured under a range of levels of pH. (Scaling exponents are presented $\pm$ s.e., with 95 per cent confidence interval in parentheses.)

pH		
acclimation	measurement	allometric equation
5.0	5.0	$0.14 \times \text{mass}^{0.42 \pm 0.09(0.23-0.61)}$
5.0	8.5	$0.13 \times \text{mass}^{0.39 \pm 0.09(0.21-0.58)}$
8.5	8.5	$0.18 \times \text{mass}^{0.78 \pm 0.12(0.54-1.02)}$
8.5	5.0	$0.08 \times \text{mass}^{0.67 \pm 0.11(0.46-0.89)}$

of the size-dependent variation in MR, and many avenues remain open for further investigation of the mechanistic basis of the variation in metabolic allometry observed here. Metabolic depression is a common response to stressful conditions (Guppy & Withers 1999), and many, but not all, fishes show a reduction in MR following exposure to low pH (e.g. Hargis 1975; Ultsch *et al.* 1980). The critical partial pressure of O_2 for MR increases and aerobic scope decreases upon acidification (Ultsch *et al.* 1980), and chronic acid exposure induces morphological changes including epidermal mucous cell hyperplasia (Zuchelkowski *et al.* 1981), chloride cell hyperplasia and structural modification (Leino & McCormick 1984), and gill remodelling that resembles the very rapid changes that occur following exposure to high temperature or hypoxia (Plonka & Neff 1969; McCormick *et al.* 1989; Sollid & Nilsson 2006). Any of these changes are potentially size-dependent and might therefore contribute to the variation in metabolic allometry described here.

The present study demonstrates a significant effect of an environmental variable on metabolic allometry, suggesting that theoretical studies which predict an invariant value of the allometric scaling exponent need to be expanded to accommodate environmental influences on the allometry of MR. Manipulations of metabolic allometry, such as the one presented in this study, represent powerful and as yet underused tools to understand the factors that constrain and influence the allometry of MR.

All experimental procedures were approved by the University of Queensland NEWMA Animal Ethics Committee (S1B/070/0/UQECR).

- Allen, A. P. & Gillooly, J. F. 2009 Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling. *Ecol. Lett.* **12**, 369–384. (doi:10.1111/j.1461-0248.2009.01302.x)
- Alton, L. A., White, C. R. & Seymour, R. S. 2007 Effect of aerial oxygen content on bimodal gas exchange and air-breathing behaviour in *Trichogaster leeri*. *J. Exp. Biol.* **210**, 2311–2319. (doi:10.1242/jeb.02778)
- Bayly, I. A. E., Ebsworth, E. P. & Wan, H. F. 1975 Studies on the lakes of Fraser Island, Queensland. *Aust. J. Mar. Freshw. Res.* **26**, 1–13. (doi:10.1071/MF9750001)
- Dodds, P. S., Rothman, D. H. & Weitz, J. S. 2001 Re-examination of the '3/4-law' of metabolism. *J. Theoret. Biol.* **209**, 9–27. (doi:10.1006/jtbi.2000.2238)
- Frappell, P. B. 2008 Ontogeny and allometry of metabolic rate and ventilation in the marsupial: matching supply and demand from ectothermy to endothermy. *Comp. Biochem. Physiol. A* **150**, 181–188. (doi:10.1016/j.cbpa.2008.02.017)
- Glazier, D. S. 2005 Beyond the '3/4-power law': variation in the intra- and interspecific scaling of metabolic rate in animals. *Biol. Rev.* **80**, 1–52. (doi:10.1017/S1464793105006834)
- Glazier, D. S. 2008 Effects of metabolic level on the body size scaling of metabolic rate in birds and mammals. *Proc. R. Soc. B* **22**, 1405–1410. (doi:10.1098/rspb.2008.0118)
- Guppy, M. & Withers, P. C. 1999 Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biol. Rev.* **74**, 1–40. (doi:10.1017/S0006323198005258)
- Hargis, J. R. 1975 Ventilation and metabolic rate of young rainbow trout (*Salmo gairdneri*) exposed to sublethal environmental pH. *J. Exp. Zool.* **196**, 39–44. (doi:10.1002/jez.1401960105)
- Jeyasingh, P. D. 2007 Plasticity in metabolic allometry: the role of dietary stoichiometry. *Ecol. Lett.* **10**, 282–289. (doi:10.1111/j.1461-0248.2007.01023.x)
- Killen, S. S., Costa, I., Brown, J. A. & Gamperl, A. K. 2007 Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. *Proc. R. Soc. B* **274**, 431–438. (doi:10.1098/rspb.2006.3741)
- Kleiber, M. 1932 Body size and metabolism. *Hilgardia* **6**, 315–353.
- Leino, R. L. & McCormick, J. H. 1984 Morphological and morphometrical changes in chloride cells of the gills of *Pimephales promelas* after chronic exposure to acid water. *Cell Tissue Res.* **236**, 121–128. (doi:10.1007/BF00216521)
- Leivestad, H. & Muniz, I. P. 1976 Fish kill at low pH in a Norwegian river. *Nature* **259**, 391–392. (doi:10.1038/259391a0)
- McCormick, J. H., Jensen, K. M. & Leino, R. L. 1989 Survival, blood osmolality, and gill morphology of juvenile yellow perch, rock bass, black crappie, and largemouth bass exposed to acidified soft water. *Trans. Am. Fish. Soc.* **118**, 386–399. (doi:10.1577/1548-8659(1989)118<0386:SBOAGM>2.3.CO;2)
- McWilliams, P. G. 1980 Effects of pH on sodium uptake in Norwegian brown trout (*Salmo trutta*) from an acid river. *J. Exp. Biol.* **88**, 259–267.
- Morlon, H. et al. 2009 Taking species abundance distributions beyond individuals. *Ecol. Lett.* **12**, 488–501. (doi:10.1111/j.1461-0248.2009.01318.x)
- Moses, M. E., Hou, C., Woodruff, W. H., West, G. B., Nekola, J. C., Zuo, W. & Brown, J. H. 2008 Revisiting a model of ontogenetic growth: estimating model parameters from theory and data. *Am. Nat.* **171**, 632–645. (doi:10.1086/587073)
- Ohlberger, J., Staaks, G. & Hölker, F. 2007 Effects of temperature, swimming speed and body mass on standard and active metabolic rate in vendace (*Coregonus albula*). *J. Comp. Physiol. B* **177**, 905–916. (doi:10.1007/s00360-007-0189-9)
- Packer, R. K. & Dunson, W. A. 1970 Effects of low environmental pH on blood pH and sodium balance of brook trout. *J. Exp. Zool.* **174**, 65–72. (doi:10.1002/jez.1401740107)
- Plonka, A. C. & Neff, W. H. 1969 Mucopolysaccharide histochemistry of gill epithelial secretions in brook trout exposed to acid pH. *Proc. Pennsylvania Acad. Sci.* **43**, 53–55.
- Riggs, A. 1960 The nature and significance of the Bohr effect in mammalian hemoglobins. *J. Gen. Physiol.* **43**, 737–752. (doi:10.1085/jgp.43.4.737)
- Savage, V. M., Deeds, E. J. & Fontana, W. 2008 Sizing up allometric scaling theory. *PLoS Comput. Biol.* **4**, e1000171. (doi:10.1371/journal.pcbi.1000171)
- Schmidt-Nielsen, K. & Larimer, J. L. 1958 Oxygen dissociation curves of mammalian blood in relation to body size. *Am. J. Physiol.* **195**, 424–428.
- Sollid, J. & Nilsson, G. E. 2006 Plasticity of respiratory structures: adaptive remodeling of fish gills induced by ambient oxygen and temperature. *Respir. Physiol. Neurobiol.* **154**, 241–251. (doi:10.1016/j.resp.2006.02.006)
- Ultsch, G. R. & Gros, G. 1979 Mucus as a diffusion barrier to oxygen: possible role in O₂ uptake at low pH in carp (*Cyprinus carpio*) gills. *Comp. Biochem. Physiol. A* **62**, 685–689. (doi:10.1016/0300-9629(79)90125-7)
- Ultsch, G. R., Ott, M. E. & Heisler, N. 1980 Standard metabolic rate, critical oxygen tension, and aerobic scope for spontaneous activity of trout (*Salmo gairdneri*) and carp (*Cyprinus carpio*) in acidified water. *Comp. Biochem. Physiol. A* **67**, 329–335. (doi:10.1016/S0300-9629(80)80004-1)
- Westfall, B. A. 1945 Coagulation film anoxia in fishes. *Ecology* **26**, 283–287. (doi:10.2307/1932407)
- White, C. R., Cassey, P. & Blackburn, T. M. 2007 Allometric exponents do not support a universal metabolic allometry. *Ecology* **88**, 315–323. (doi:10.1890/05-1883)
- Zuchelkowski, E. M., Lantz, R. C. & Hinton, D. E. 1981 Effects of acid-stress in epidermal mucous cells of the brown bullhead *Ictalurus nebulosus* (Leseur): a morphometric study. *Anat. Rec.* **200**, 33–39. (doi:10.1002/ar.1092000104)