

## LETTER

# The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature

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## Abstract

Metabolic energy fuels all biological processes, and therefore theories that explain the scaling of metabolic rate with body mass potentially have great predictive power in ecology. A new model, that could improve this predictive power, postulates that the metabolic scaling exponent ( $b$ ) varies between  $2/3$  and  $1$ , and is inversely related to the elevation of the intraspecific scaling relationship (metabolic level,  $L$ ), which in turn varies systematically among species in response to various ecological factors. We test these predictions by examining the effects of lifestyle, swimming mode and temperature on intraspecific scaling of resting metabolic rate among 89 species of teleost fish. As predicted,  $b$  decreased as  $L$  increased with temperature, and with shifts in lifestyle from bathyal and benthic to benthopelagic to pelagic. This effect of lifestyle on  $b$  may be related to varying amounts of energetically expensive tissues associated with different capacities for swimming during predator–prey interactions.

## Keywords

Body size, ecology, ecophysiology, metabolism, predator–prey, swimming mode, teleosts.

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## INTRODUCTION

Metabolism enables organisms to transform energy and materials from the environment into biological structures and functions. The metabolic rate and ecology of organisms should therefore be interrelated. On the one hand, an organism's ecosystem will be influenced by the energetic demands of that individual. On the other hand, ecological factors, acting either directly on physiology or as selective pressures, will affect an organism's growth, locomotion and reproduction, and hence its energetic demands. The complex interactions between ecology and metabolism may influence life-history evolution, and affect numerous biological and ecological rates (e.g. Brown *et al.* 2004; West & Brown 2005). Therefore, understanding and predicting the influences on meta-

bolic rates are of high importance throughout much of ecology.

Attempts to understand the influence of body size on metabolic rate has a long history, with proposed explanations emphasizing various combinations of physical principles and adaptive variation (reviews in Agutter & Wheatley 2004; Glazier 2005; da Silva *et al.* 2006). The relationship between resting metabolic rate ( $R$ ) and body mass ( $M$ ) can generally be described by the equation  $R = aM^b$ , where  $a$  is the scaling coefficient and  $b$  is the scaling exponent or slope of the allometric plot of  $\log R$  vs.  $\log M$ . The scaling coefficient  $a$  varies widely among taxa (White *et al.* 2006; Seibel 2007), whereas the scaling exponent  $b$  has traditionally been assumed to be fixed at  $c. 0.75$  across all organisms, thus representing a universal scaling 'law' (e.g. Hemmingsen 1960; Savage *et al.* 2004). Recent doubts about the existence

of such a law arise from large variation in  $b$  recently reported for animals and plants, both within and between species (Bokma 2004; Glazier 2005; White *et al.* 2006; Seibel 2007; Makarieva *et al.* 2008). A key question is to what extent is this variation just statistical 'noise' about an average scaling relationship, compared with systematic variation that could depend on specific ecological or biological factors. Indeed, if variation in  $b$  is systematic, quantifying the extent of this variation and investigating its causes will be crucial for making accurate, energy-based predictions of ecological processes. In this paper, we test the alternative hypotheses that  $b$  varies randomly around an average value vs. that it has no single average value, but varies systematically with respect to specific environmental and lifestyle factors.

The recently proposed 'metabolic level boundaries hypothesis' (MLB hypothesis; Glazier 2005, 2008, 2009a) offers a radically different approach from traditional metabolic scaling theories that examine the causes of average scaling exponents. Instead, the MLB hypothesis aims to explain variation in the scaling of metabolic rate with body size. Building on the work of Kooijman (2000) and others (e.g. Hemmingsen 1960), the MLB hypothesis assumes that the scaling of metabolic rate in organisms may be limited by two boundary constraints: (1) surface-area limits on fluxes of resources, wastes and heat; and (2) mass or volume limits on resting tissue maintenance costs or on power production during strenuous activity. The relative importance of these two constraints will depend on the elevation of the scaling relationship of metabolic rate with body size (i.e. 'metabolic level'). As a result,  $b$  in relatively inactive organisms (i.e. those in a dormant or resting state or engaged in relatively inexpensive routine activities) should vary inversely with metabolic level, whereas those engaged in non-sustainable strenuous activities should show positive correlations between  $b$  and metabolic level. In organisms for which the costs of maintenance and routine activity are high, the scaling of metabolic rate with body size should be primarily limited by fluxes of resources and wastes across surfaces (scaling as  $M^{2/3}$  for external surfaces or as  $M^{3/4}$  if primarily limited by internal transport: West *et al.* 1997, though there is uncertainty about this latter prediction; e.g. Apol *et al.* 2008). In contrast, for organisms in which these metabolic costs are low and amply met by surface-dependent and internal transport processes, the scaling of metabolic rate should be closer to being directly proportional to body mass (i.e. scaling as  $M^1$ ; where the relationship is more related to volume limits on the metabolic output required for tissue maintenance). This hypothesis agrees with the general observation that  $b$  for ectothermic vertebrates (e.g. mean values for  $b$  are often  $> 0.80$ ; Bokma 2004; Glazier 2005; White *et al.* 2006) tends to be higher than that for endotherms which spend much more energy on heat production (mean values for  $b$  typically

range from 0.66 to 0.75; Glazier 2005; White *et al.* 2006; Glazier 2008). The MLB hypothesis has also successfully predicted significant covariation of  $b$  with different levels of physical activity (Glazier 2008, 2009a,b).

The MLB hypothesis could provide a mechanistic link between the ecology of organisms and the scaling of metabolism with body size because according to this model, any ecological factor influencing resting or routine metabolic rate could potentially affect  $b$ . For instance, factors such as predation influence animal activity level (Lima & Dill 1990; Killen & Brown 2006), reproductive investment (Reznick *et al.* 1996) and growth rate (Biro *et al.* 2004; Killen & Brown 2006), and thus have the potential to affect metabolic rate. Species with different lifestyles, or which live in different habitats, will have different requirements for foraging and predator avoidance, and may therefore evolve differences in metabolic level that influence their patterns of intraspecific metabolic scaling. For example, marine animals living in the deep ocean have lower mass- and temperature-specific resting metabolic rates than those in more shallow waters, possibly because of lower activity requirements for foraging and predator avoidance (Childress 1995; Seibel & Drazen 2007). In accordance with the MLB hypothesis, such differences in metabolism could cause variation in the scaling of metabolic rate among species with differing lifestyles. This would suggest a three-way link between ecological selection pressures, metabolic level and the scaling of metabolic rate with body size (Glazier 2005, 2009a).

We explore these potential interactions in teleost fishes, a group that is well-studied, occupies various ecological niches, and has body-mass ranges for individual species that span up to eight orders of magnitude during ontogeny. In addition, their diverse modes of swimming may be related to both ecological factors and metabolic level, and hence patterns of metabolic scaling. These characteristics make fishes ideal for investigating ecological influences on metabolic scaling, as opposed to the traditional focus on body-design factors.

## MATERIALS AND METHODS

### Literature survey

Data on the relationship between resting aerobic metabolic rate (estimated from oxygen consumption) and body mass were collected from the literature for 89 species of teleost fish (see Appendix S1). We supplemented the data set of Clarke & Johnston (1999) with additional data. Like Clarke & Johnston (1999), we only accepted data (with a few exceptions, justified in Appendix S1), if measurements had been performed on quietly resting, post-larval animals, that had been fasted prior to measurement and that were

measured with no additional stressors (e.g. no salinity or temperature stress, given > 24 h for acclimation to respirometers, etc.). Finally, studies were excluded if experimental temperatures were outside the range experienced by the species in nature, or if metabolic rates were not measured over a suitable body-mass range (see Appendix S1 for a more detailed explanation of data acceptance criteria). Only one data set per species was used to avoid giving undue weight to species with multiple studies (Clarke & Johnston 1999). In cases where multiple data sets were available for a given species, priority was given to the study performed closest to the middle of the species' natural temperature range, followed by measurements covering the widest body-mass range.

The metabolic rate of resting, fasted fish, with no additional stressors, as required for inclusion in the present study, was deemed 'standard' metabolic rate (SMR; that required for maintenance alone) by some authors, and 'routine' metabolic rate (RMR; that required for maintenance plus random activity) by others, probably due to differences among species in the amount of random activity displayed in respirometers. However, after adjustment for differences in measurement temperature (see Data and statistical analyses) we detected no effect of author classification of metabolic state (i.e. SMR vs. RMR) on  $b$  or metabolic level,  $L$  (unpaired  $t$ -tests,  $P = 0.095$  for  $b$ ;  $P = 0.843$  for  $L$ ; see Data and statistical analyses for description of  $L$ ). In addition, when metabolic state was included in a two-way ANOVA with lifestyle or swimming mode, no significant interactions or main effects of metabolism classification were observed. As such, all data for  $b$  and  $L$  were analysed as a whole (not differentiated as either SMR or RMR).

Metabolic scaling exponents ( $b$ ) and metabolic level ( $L$ ) (see Data and statistical analyses) were related to lifestyle using the classification for each species obtained from <http://www.FishBase.org> (Froese & Pauly 2008). Four main lifestyle groupings were used in this analysis: 'pelagic' ( $n = 15$ ), 'benthopelagic' ( $n = 21$ ), 'benthic' ( $n = 44$ ) and 'bathyal' ( $n = 9$ ). Pelagic species live in the open water column and generally feed near the water surface. Benthopelagic species live and feed near (but not on) the bottom, sometimes associating with midwaters or even surface waters depending on depth. Benthic species live on the bottom in direct contact with the substrate. Bathyal species live in the deep sea, at  $c.$  1000–5000 m in depth.

Both  $b$  and  $L$  were also analysed in relation to swimming mode, as classified by Breder (1926), with the classification for each species obtained, where available, from <http://www.FishBase.org>. Breder (1926) classified fish species as either anguilliform ( $n = 19$  in our data set), subcarangiform ( $n = 19$ ), carangiform ( $n = 7$ ) or thunniform ( $n = 4$ ) swimmers, representing a transition from the use of body

undulations for propulsion (anguilliform), to the use of the caudal fin to generate thrust (thunniform; see Lindsey 1978 for a detailed description).

## Data and statistical analyses

We attempted to define a mathematically coherent and biologically relevant measure of metabolic level ( $L$ ) that can be universally adopted in tests that quantitatively relate  $L$  to estimates of  $b$ . The scaling coefficient  $a$  has previously been used as an indicator of  $L$  in allometric scaling relationships (Prosser 1973; Glazier 2009a,c), but is problematic when values of  $b$  are variable because  $a$  is not independent of  $b$ . For example, in a log–log plot of metabolic rate vs. body mass, any change in the slope ( $b$ ) will necessarily change the intercept ( $a$ ). Furthermore, a dimensional analysis of the power function relating metabolic rate to body mass shows that  $a$  has units of energy consumed per unit time per unit body mass <sup>$b$</sup>  (Xiao 1998), further illustrating an interdependence between  $a$  and  $b$ . The lack of independence of  $a$  from the exponent  $b$  applies to intercept estimates of  $L$  at all body masses except that representing the midpoint of the linear allometric regression relationship. Only at this point will variation in  $b$  not affect the intercept estimate of  $L$  (Glazier 2009a,c). Therefore, we define  $L$  as the mass-specific metabolic rate estimated at the body mass corresponding to the midpoint of the allometric relationship (the 'midpoint body mass'). Another problem associated with using  $a$  to represent  $L$  is that, in a log–log plot of metabolic rate vs. body mass, the exact value of  $a$  will depend on the units used to represent body mass. Again, however, the estimate of  $L$  at the midpoint body mass does not have this problem.

All metabolic rate data were converted to  $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$  for the calculation of  $L$ . To examine temperature effects, Arrhenius relationships were investigated for  $b$ , and  $\ln L$  against  $1/kT$ , where  $T$  is temperature in Kelvin and  $k$  is the Boltzmann constant ( $8.62 \times 10^{-5} \text{ eV K}^{-1}$ ). The Arrhenius activation energies ( $E_a$ , in eV) were calculated as the absolute value of the linear slope of this relationship. The  $Q_{10}$  value (0–30 °C) for  $L$  was estimated from a plot of  $\ln L$  vs. temperature in °C (Fig. S1) using the equation:

$$(R_{30}/R_0)^{(10/(30-0))} \quad (1)$$

where  $R_{30}$  and  $R_0$  are metabolic rates at 30 and 0 °C estimated from the fit of ordinary least squares regression to the data [appropriate in this case because most of the error will be in the  $y$ -variable ( $\ln L$ ), rather than the  $x$ -variable (temperature, or  $1/kT$ )]. ANCOVA was used to examine the effects of temperature (used as a covariate), lifestyle or swimming mode, and the interaction of these factors ('temperature  $\times$  lifestyle' interaction or 'temperature  $\times$  swimming mode' interaction). The combined effects of temperature

and lifestyle were further analysed by placing all species into either a high ( $> 15\text{ }^{\circ}\text{C}$ ) or low ( $\leq 15\text{ }^{\circ}\text{C}$ ) temperature 'bin'. The  $b$  and  $L$  values for those species measured between  $-1.5$  and  $15\text{ }^{\circ}\text{C}$  were then adjusted to  $5\text{ }^{\circ}\text{C}$  (by expressing the residuals of the Arrhenius plots for  $b$  and  $\ln L_{\text{mid}}$ , relative to fitted equation values at the equivalent of  $5\text{ }^{\circ}\text{C}$ ), while those measured between  $16$  and  $37\text{ }^{\circ}\text{C}$  were adjusted to  $25\text{ }^{\circ}\text{C}$ . Correlations between mean  $b$  and mean  $L$  across lifestyle categories at the different adjustment temperatures were analysed by Reduced Major Axis (RMA) regression using RMA software version 1.17 (Bohonak 2002; available at <http://www.bio.sdsu.edu/pub/andy/rma.html>).

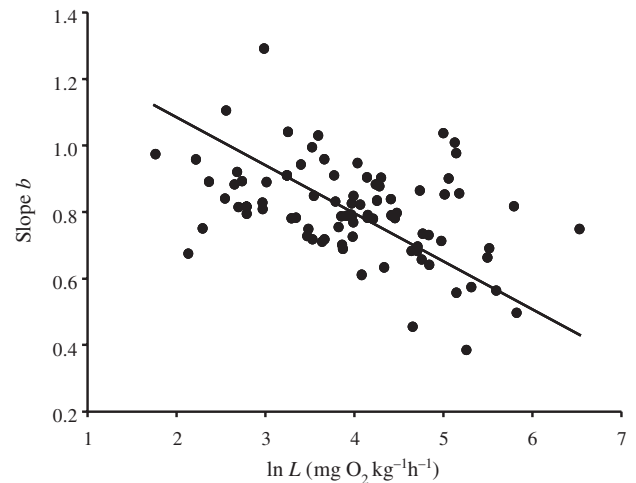
To more closely examine the effects of lifestyle and swimming mode alone on  $b$  and  $L$ , we adjusted all data to a common temperature of  $15\text{ }^{\circ}\text{C}$  (using residuals of the Arrhenius plots as previously described). Attempts to correct for the effects of temperature on metabolic rate in ectotherms can be problematic as extrapolation beyond the thermal optima of species may produce inaccurate estimates of metabolic rate. We minimized these problems, however, by correcting metabolic rate data to  $15\text{ }^{\circ}\text{C}$  – an intermediate temperature relative to the thermal ranges of most species included in the present study. We then analysed differences in  $b$  and  $L$  across lifestyle and swimming mode classifications using pairwise randomization tests (Edgington 1995). The resulting  $P$ -value was then adjusted using a sequential Bonferroni procedure (Bonferroni-Holm adjustment) to account for the multiple pairwise comparisons. Correlations between mean  $b$  and mean  $L$  across lifestyle and swimming mode categories after adjustment to  $15\text{ }^{\circ}\text{C}$  were analysed using RMA regression.

Statistical analyses were performed using Minitab (v. 13.0; Minitab Inc., State College, Pennsylvania, USA), Statview (v. 5.0; SAS Institute Inc., Cary, North Carolina, USA), and the Resampling Stats plug-in (v. 3.2; Resampling Stats Inc., Arlington, Virginia, USA) for Microsoft Office Excel 2003 (Microsoft Corp., Redmond, Washington, USA). The assumptions of ANCOVA were verified using Kolmogorov–Smirnov tests (to examine normality of residuals), and residual-fit plots and Levene's tests (homogeneity of variances). The level of significance ( $\alpha$ ) for all tests was  $P = 0.05$ . Data in text and on bar graphs are presented as means  $\pm$  SEM.

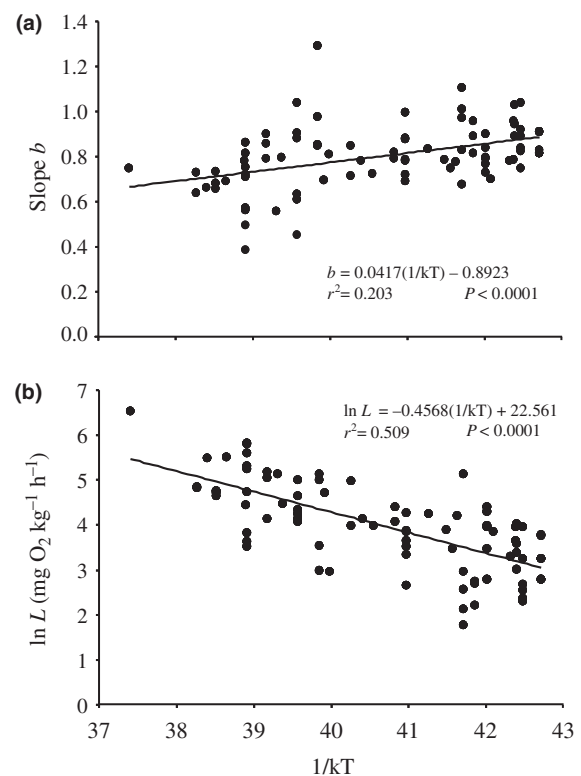
## RESULTS

Before doing a more detailed analysis of lifestyle, swimming mode and temperature effects, the  $b$ -values for all 89 species were found to show a negative correlation with  $L$  (Fig. 1; RMA regression,  $r^2 = 0.18$ ).

Measurement temperature affected  $b$  and  $L$  (ANCOVA,  $P < 0.05$ ) with an Arrhenius activation energy ( $E_a$ ) of  $0.457(\pm 0.048)\text{ eV}$  for  $L$  (Fig. 1). Values for  $b$  generally



**Figure 1** The relationship between  $b$  and  $L$  among the 89 species used in the present study [RMA regression,  $r^2 = 0.18$ ,  $b = -0.145(95\% \text{ CI: } -0.1725, -0.1166)L + 1.377 (1.263, 1.491)$ ].



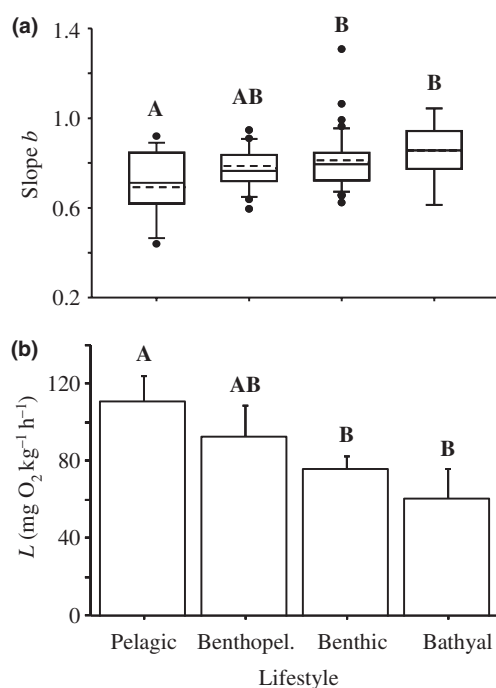
**Figure 2** Arrhenius plots illustrating the relationship between temperature and: (a) the scaling exponent  $b$ ; and (b) the metabolic level, estimated as the metabolic rate at the midpoint of the regression line used to determine  $b$  for each species of the current study ( $L$ ).  $T$  is temperature in Kelvin and  $k$  is the Boltzmann constant ( $8.62 \times 10^{-5}\text{ eV K}^{-1}$ ). The Arrhenius activation energies ( $E_a$ , in eV) were calculated as the absolute value of the linear slope of these relationships.

decreased with temperature (Fig. 2; Fig. S1), and the  $Q_{10}$  value for  $L$  was 1.91 over the temperature range 0–30 °C (Fig. S1). The effect of temperature on  $L$  and  $b$  was not significantly different among the individual lifestyle and swimming mode groupings (slopes were not significantly different).

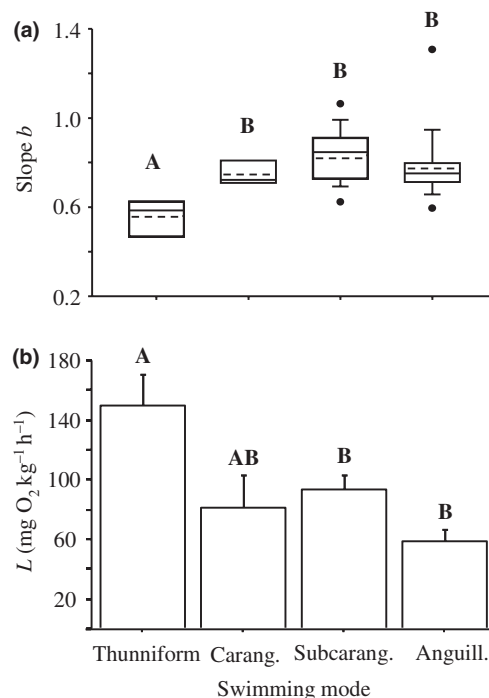
Before adjusting for temperature effects, both lifestyle and swimming mode had significant main effects on  $b$  and  $L$  (ANCOVA,  $P < 0.05$ ). After adjusting for temperature effects by using residuals of the Arrhenius plots (expressed relative to a common temperature of 15 °C), lifestyle and swimming mode still had a significant effect on  $L$ . In general,  $L$  decreased across pelagic, benthopelagic, benthic and bathyal lifestyles (Fig. 3). At the extreme ends of this spectrum, the mean  $L$  for the pelagic group ( $111.54 \pm 13.39$  mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>) was 46% higher than for benthic fishes ( $76.32 \pm 6.66$  mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>; randomization test,  $P = 0.046$ ) and 82% higher than for the bathyal fishes ( $61.37 \pm 15.26$  mg

O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>; randomization test,  $P = 0.045$ ). Mean values for  $b$  after adjustment to 15 °C showed an increasing trend across pelagic (mean  $b = 0.698 \pm 0.036$ ), benthopelagic ( $0.776 \pm 0.02$ ), benthic ( $0.802 \pm 0.018$ ) and bathyal ( $0.856 \pm 0.042$ ) lifestyles (Fig. 3), with the pelagic group having a significantly lower  $b$ -value than that of both the benthic and bathyal groups (randomization tests,  $P = 0.015$  for pelagic vs. benthic, and  $P = 0.0265$  for pelagic vs. bathyal). It should also be noted that the same trends were observed even when several different methods for estimating  $L$  (albeit less appropriate, see Materials and methods) were used, specifically the scaling coefficient  $a$  and the predicted oxygen consumption at 350 g for each species, which was close to the mean midpoint mass of the species used in this study (results not presented).

Swimming mode had a significant effect on  $L$ , generally increasing throughout the transition across anguilliform, subcarangiform, carangiform and thunniform swimming

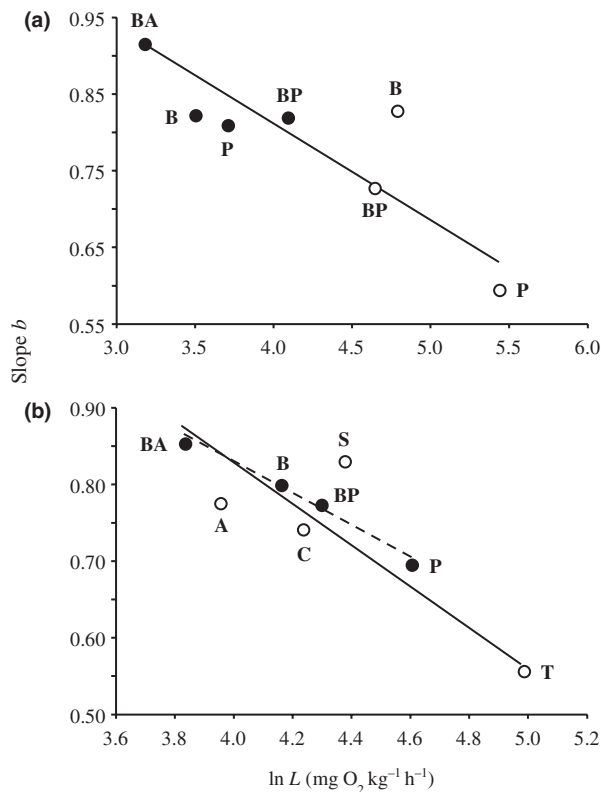


**Figure 3** The effect of lifestyle on: (a) the scaling exponent  $b$ ; and (b) the metabolic level, estimated as the metabolic rate at the midpoint of the regression line used to determine  $b$  for each species of the current study ( $L$ ). Significant differences between lifestyle groupings are indicated by different letters (randomization tests,  $P < 0.05$  after sequential Bonferroni adjustment). For (a), the boundary of each box closest to zero indicates the 25th percentile, the solid line within each box marks the median, the dashed line represents the mean, and the boundary of the box farthest from zero indicates the 75th percentile. The error bars above and below each box indicate the 90th and 10th percentiles respectively. For (b), values are means  $\pm$  SEM.



**Figure 4** The effect of swimming mode on: (a) the scaling exponent  $b$ ; and (b) the metabolic level, estimated as the metabolic rate at the midpoint of the regression line used to determine  $b$  for each species of the current study ( $L$ ). Significant differences between swimming mode groupings are indicated by different letters (randomization tests,  $P < 0.05$  after sequential Bonferroni adjustment). For (a), the boundary of each box closest to zero indicates the 25th percentile, the solid line within each box marks the median, the dashed line represents the mean and the boundary of the box farthest from zero indicates the 75th percentile. The error bars above and below each box indicate the 90th and 10th percentiles respectively. For (b), values are means  $\pm$  SEM.





**Figure 5** Reduced major axis regressions between: (a)  $b$  and  $L$  for the different lifestyle categories used in the present study (BA, bathyal; B, benthic; BP, benthopelagic; P, pelagic), with data adjusted to either 5 °C (for species measured between –1.5 °C and 15 °C; filled circles) or 25 °C (for species measured between 16 °C and 37 °C; empty circles);  $b = -0.127$  (95% CI:  $-0.20485, -0.04813$ ) $L + 1.320$ (0.987, 1.653),  $r^2 = 0.71$ ; and between (b)  $b$  and  $L$  for lifestyle and swimming mode classifications after adjustment of all data to 15 °C. Filled circles represent mean values for  $L$  and  $b$  for the various lifestyle groupings (BA, bathyal; B, benthic; BP, benthopelagic; P, pelagic); open circles represent mean values for swimming mode groupings (A, anguilliform; S, subcarangiform; C, carangiform; T, thunniform). The dashed line represents the regression for lifestyle values ( $b = -0.206$ (0.2835,  $-0.1283$ ) $L + 1.652$ (1.324, 1.980),  $r^2 = 0.98$ ); the solid line represents the regression for swimming mode values ( $b = -0.273$ ( $-0.7551, 0.2092$ ) $L + 1.021$ ( $-0.196, 4.046$ ),  $r^2 = 0.66$ ).

modes (Fig. 4). At the extremes after adjustment to 15 °C,  $L$  for resting fish having the thunniform swimming mode ( $149.29 \pm 20.02 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ) was 2.6 times higher than that of those having the anguilliform mode ( $58.37 \pm 6.91 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ; randomization test,  $P = 0.003$ ). The mean values for  $b$  were not significantly different among the anguilliform ( $0.778 \pm 0.034$ ), subcarangiform ( $0.833 \pm 0.026$ ) and carangiform ( $0.744 \pm 0.027$ ) swimming modes, but  $b$  for the thunniform swimming mode ( $0.559 \pm 0.043$ ) was significantly lower than each of these groupings

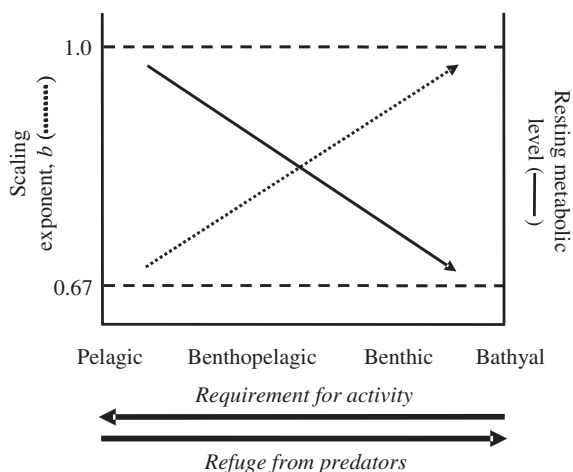
(randomization tests,  $P = 0.0006$  for thunniform vs. anguilliform;  $P = 0.0008$  for thunniform vs. subcarangiform; and  $P = 0.0006$  for thunniform vs. carangiform).

Plotting the mean value of  $b$  in each lifestyle grouping against the respective mean values for  $L$  after the binned temperature correction (to either 5 or 25 °C) resulted in a strong negative correlation between  $b$  and  $L$  (RMA regression,  $r^2$  for  $b$  vs.  $L = 0.71$ ; Fig. 5a). This analysis was not performed with swimming mode classifications due to greatly decreased sample sizes for each swimming mode after the sorting of data into the temperature bins above and below 15 °C. After adjustment of all data to a common temperature of 15 °C, mean values for  $b$  were negatively correlated with mean  $L$  values across lifestyle and also swimming mode classifications (Fig. 5b). The  $r^2$  values for RMA regressions of the mean values for  $b$  for each of the lifestyle and swimming mode groupings vs. the corresponding mean values for  $L$  were large in both cases ( $r^2 = 0.98$  for lifestyle analysis;  $r^2 = 0.66$  for swimming mode analysis), thus indicating a consistently large effect size.

## DISCUSSION

As predicted by the MLB hypothesis, the intraspecific scaling of resting metabolic rate with body mass in 89 teleost fishes shows substantial and systematic variation in  $b$  with regard to lifestyle, swimming mode and ambient temperature and that is inversely related to  $L$ . Even after adjustment for differences in measurement temperature, the variation in intraspecific scaling exponents across lifestyles for fishes is considerable, with the difference in the mean values of  $b$  for pelagic ( $0.698 \pm 0.036$ ) and bathyal fishes ( $0.856 \pm 0.042$ ) spanning 48% of the hypothesized possible range (0.67–1.0; Kooijman 2000; Glazier 2005, 2009a).

The observed inverse relationship between  $b$  and  $L$  across the lifestyle groupings also suggests that ecology affects intraspecific metabolic scaling in fishes through its effects on the metabolic rate needed for maintenance or routine activity (Fig. 6). It has previously been theorized that differences in the amount of light and structural complexity among marine habitats can influence maximal metabolic rates (MMR) by affecting the nature of predator–prey interactions (Childress 1995; Seibel & Drazen 2007). For example, across the spectrum of habitats associated with pelagic, benthopelagic, benthic and bathyal lifestyles, there is a decreasing reliance on visual predation and active pursuit of prey during foraging. In the pelagic realm, there is little physical structure to provide refuge from predators, and so many prey species must be able to move quickly to avoid attacks or capture prey themselves. Benthopelagic species associate with both open water and the substrate, and therefore have some access to complex structures for either hiding from predators or finding prey items without long chases, yet will also be



**Figure 6** Schematic of the interactions between lifestyle, metabolic level and the scaling of metabolic rate with body size in fishes. Across pelagic, benthopelagic, benthic, and bathyal lifestyle groupings there is an increase in the refuge available for avoiding visual predation (through either increased habitat complexity or a general decrease in light levels). This results in reduced selection for swimming capacity, and lowered resting metabolic rates in individuals (and a lowered metabolic level across size ranges). According to the 'metabolic level boundaries hypothesis', these differences in metabolic level across lifestyles will cause variation in the scaling exponent  $b$ . Lifestyles routinely requiring high locomotory capacity (e.g. pelagic species) are predicted to have a high metabolic level, and increases in metabolic rate with body size in these species will therefore be more constrained by surface area limitations on fuel acquisition and waste removal ( $b$  will be closer to 0.67). Lifestyles not routinely requiring high locomotory capacity (e.g. benthic and bathyal species) are predicted to have a lower metabolic level, and so metabolic scaling with body mass will mainly be constrained by volume-related limits on the resting metabolic output required for tissue maintenance ( $b$  will be closer to 1.0).

subject to high-activity predator–prey interactions in the water column. In contrast to pelagic and benthopelagic species, benthic species live within complex habitat (amongst reefs, rocks, vegetation), and usually rely on ambush foraging to capture prey instead of active pursuits. Finally, light levels are so low at bathyal depths that visual predation and the high-speed pursuit of prey is probably nonexistent (Drzen 2007; Seibel & Drzen 2007). The end result of this spectrum of activity requirements is a gradient of decreasing 'athleticism' from pelagic and benthopelagic to benthic and bathyal species (Seibel & Drzen 2007).

How might such differences in athleticism across lifestyles lead to variation in  $b$  for resting metabolic rate? Across animal taxa, species with increased maximal metabolic rates tend to also have increased resting metabolic rates (Taigen 1983; Walton 1993; Reinhold 1999). High athletic performance typically requires increased skeletal

muscle mass (Weibel *et al.* 2004), heart size (Bishop 1997) and respiratory surface area (Muir & Hughes 1969), all of which may contribute to increased costs in animals while at rest due to the increased ATP expenditure required for tissue maintenance (e.g. the production and replacement of proteins, the synthesis of nitrogenous waste molecules) and ion balance (e.g. energy expenditure of  $\text{Na}^+/\text{K}^+$ - and  $\text{Ca}^{2+}$ -ATPases). Furthermore, athleticism is positively correlated with muscle mitochondrial density (Hoppeler *et al.* 1987), which could lead to an increase in the total energy expenditure owing to proton leakage across mitochondrial membranes (Brand 1990). According to the MLB hypothesis, these elevated maintenance costs should enhance the importance of surface-area limitations (e.g. respiratory surface area, total capillary surface area) on sustainable metabolic rate with body size, thus causing a decrease in values of  $b$  even in resting animals (especially at high body temperatures) that have metabolic rates below that which is maximally possible during non-sustainable vigorous exercise. Indeed, among the lifestyle groupings in the present study, pelagic species have the highest requirement for swimming ability, and accordingly, had the highest mean value for  $L$  and lowest mean value for  $b$ . The fact that the observed variation in  $b$  follows a predictable pattern across lifestyle groupings provides strong evidence for the existence of real systematic variation in  $b$ , and suggests that this variation is not merely statistical 'noise' about an average scaling exponent.

Benthic and bathyal species had the highest levels for  $b$  and the lowest values for  $L$ . This observation for bathyal species agrees with previous reports showing that deeper-living animals generally have lower metabolic rates (Childress 1995; Seibel & Drzen 2007). Despite the scarcity and potentially poorer quality of metabolic measurements for bathyal species, these results suggest that the intraspecific trends for  $b$  and  $L$  present across pelagic, benthopelagic and benthic species may also extend to bathyal species. Trends in deep-sea species also illustrate the general point that ecological selection pressures may influence the scaling of metabolic rate by affecting tissue composition. Specifically, in contrast to the increased muscle mass and mitochondrial densities observed in athletic animals, the muscle protein content of marine fishes generally decreases with depth, while muscle water content increases (Drzen 2007). This change in the functionality of muscle fibres in deep-sea fishes with depth is likely a response to relaxed selection for high swimming capacity (Drzen 2007), and probably contributes to these species displaying a relatively low metabolic rate when at rest.

The relationship between locomotory ability and metabolism is supported by our analysis of swimming mode types. Tunas and dolphinfish show several morphological and physiological adaptations for high-performance

swimming (Muir & Hughes 1969; Moyes *et al.* 1992; Bushnell & Jones 1994), and have mass-specific SMRs that are several times higher than those observed in other teleosts (Dewar & Graham 1994). In accordance with the MLB hypothesis, these thunniform swimmers display relatively low estimates for  $b$ . The three genera of tunas included within the pelagic lifestyle grouping (*Euthynnus*, *Katsuwonus* and *Thunnus*) also possess varying capacities for endothermy (Block *et al.* 1993), which increases with body mass (Graham & Dickson 2001), and so correcting for any differences in internal temperature between body sizes would likely produce intraspecific estimates of  $b$  for tuna species even lower than those used in the present study. In contrast to the thunniform swimmers, the anguilliform swimming mode was represented entirely by benthic and bathyal species and displayed relatively high  $b$  and low  $L$  values. Carangiform and subcarangiform swimming modes each show a mix of foraging and predator avoidance strategies (e.g. subcarangiform includes benthic, benthopelagic and pelagic; carangiform includes benthopelagic and pelagic), which may explain the less clear metabolic trends.

We removed the effect of temperature to detect more clearly the influence of lifestyle and swimming mode on  $b$  and  $L$ . Metabolic level increased with temperature, with  $Q_{10}$  values closely resembling that found for teleost fishes over the same temperature range by Clarke & Johnston (1999;  $Q_{10} = 1.83$ ). In addition, values for  $b$  declined with increased temperature, providing further evidence for an inverse relationship between  $b$  and  $L$ . This finding also highlights a potential link between an environmental variable (i.e. temperature) and intraspecific variation in the scaling of metabolic rate with body size in organisms. Furthermore, these results contradict the assumption of the Metabolic Theory of Ecology (Brown *et al.* 2004) that the effect of body size on metabolic rate (as represented by  $b$ ) is independent of the effect of temperature (Glazier 2005).

Besides the MLB hypothesis, there are other potential biological explanations for some of our findings. For instance, the extent to which growth is achieved by increasing either cell size or cell number has been theorized to affect both inter- and intraspecific estimates of  $b$  (Kozłowski *et al.* 2003; Chown *et al.* 2007). Moreover, this 'cell-size model' predicts a similar range for possible  $b$ -values as the MLB hypothesis, in that  $b$  should equal 0.67 if growth is achieved solely through an increase in cell size, or 1.0 if growth is due solely to an increase in cell number (and should be somewhere between these values if growth results from a mix of both of these factors). While the range of intraspecific  $b$ -values observed in the present study generally agrees with that predicted by the cell-size model, we do not know the relative importance of increasing cell size and cell number during growth for the species used in this study.

Moreover, the cell-size model does not predict the observed inverse relationship between  $L$  and  $b$ . Still, there is considerable variation in  $b$  within lifestyle groups and across temperatures that may be explained by the influence of structural factors such as cell size variation during growth.

An inverse relationship between  $L$  and  $b$  could arise independently of the mechanisms of the MLB hypothesis if both  $L$  and  $b$  were affected by a third variable such as midpoint body mass (Glazier 2009c). This idea was not supported, as midpoint body mass was not correlated with either  $L$  or  $b$  among the 89 species in the data set. Interestingly, however, the inverse relationship between  $L$  and  $b$ , coupled with an upper limit of 1.0 for  $b$ , does complement the idea that there is a minimal mass-specific metabolic rate required to maintain life (Makarieva *et al.* 2006). Assuming that there is such a minimum, species with very low mass-specific values for  $L$  should be restricted in the extent to which they can further decrease their mass-specific metabolic rate with increases in body size before encountering this minimum limit. Therefore, the scaling relationship in such species would be nearly horizontal when  $L$  is expressed on a mass-specific basis (i.e.  $b$  for whole organism metabolic rate would be nearly equal to 1.0).

The phylogenetic relatedness of species within a particular lifestyle grouping could influence the overall mean values for  $b$  and  $L$  in that group. The phylogeny of lifestyles should be driven by environmental factors, however, and so lifestyles corresponding with specific ecological niches should be closely intertwined with taxonomic affiliation. For example, the order Clupeiformes is represented entirely by pelagic species in our data set, whereas the order Pleuronectiformes is represented solely by benthic flatfishes (Appendix S1). Thus, correcting for phylogeny would largely remove the ecological effects being examined. In our data set, the only order to contain pelagic, benthopelagic, benthic and bathyal species was the Perciformes. Consistent with our other findings, the species within this order showed the same general trends for  $b$  and  $L$  as in the data set as a whole (Fig. S1).

In conclusion, our results indicate that ecological factors affect not only metabolic rates but also the intraspecific scaling of metabolic rate with body mass. Thus, the environment and ecology of species can affect patterns of intraspecific metabolic scaling, probably over evolutionary timescales in the case of lifestyle effects and temperature, and possibly over single lifetimes in the case of temperature effects. The results of this study are also in accordance with the predictions of the MLB hypothesis. While our findings offer qualitative explanations for differences in metabolic level due to lifestyle and temperature, additional research is required to propose quantitative predictions based on such ecological influences. Future work will focus on developing the mechanistic and quantitative foundations of the MLB



hypothesis, and its integration with other theories which attempt to explain variation in the scaling of metabolic rate with body mass. Further, while the present study focused solely on intraspecific scaling, the effect of ecological factors such as lifestyle on variation in interspecific scaling patterns (Glazier 2009a) is also a worthwhile avenue for future research.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

**Figure S1** The relationship between temperature in degree Celsius and  $b$  and  $L$ .

**Figure S2** The effect of lifestyle on  $b$  and  $L$  in the order Perciformes.

**Appendix S1** Data set used in the present study and selection criteria.

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