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**Allometric relationship between body mass and aerobic metabolism**  
**inzebrafish *Daniorerio***

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The relationship between body mass and metabolic rate was investigated through the assessment of active and standard metabolic rate at different life stages in zebrafish *Danio rerio* (5 day-old larvae, 2 month-old juveniles and 6 month-old adults). Scaling exponents and constants were assessed for standard ( $R_S=0.273M^{0.965}$  in  $\text{mgO}_2\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ) and active metabolic rate ( $R_A=0.799M^{0.926}$  in  $\text{mgO}_2\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ). These data provide the basis for further experiments regarding the effects of environmental factors on aerobic metabolism throughout the life cycle of this species.

Key words: allometric scaling exponent, aerobic metabolism, zebrafish

Active metabolic rate ( $R_A$ ) is the maximal aerobic metabolic rate of an organism in a highly active state and standard metabolic rate ( $R_S$ ) is the minimal metabolic rate necessary for supporting maintenance activities (e.g. ventilation) measured in resting, starved and non-maturing individual (Fry, 1947; Brett, 1964). Aerobic metabolic scope ( $R_{AS}$ ) is the difference between  $R_A$  and  $R_S$  and represents the capacity of an organism to provide oxygen to sustain energy demanding activities (Fry, 1947).  $R_{AS}$  can be influenced by a set of environmental parameters such as temperature, oxygen and salinity (Shurmann & Steffensen, 1997; Lefrançois & Claireaux, 2003; Johansen & Jones, 2011). It has therefore been used in many studies to assess the influence of environmental factors on metabolic performance and energy-demanding activities in various fish species (Fry, 1971; Priede, 1985; Claireaux & Lefrançois, 2007). It has been demonstrated, in many species however, that intrinsic parameters such as ontogeny and body size can also influence metabolism independently of any effects of environmental conditions (Clark & Johnston, 1999; Bokma, 2004; Killen, 2007, 2010; Moran & Wells, 2007).

Zebrafish *Daniorerio* (Hamilton 1822) is a small teleost species whose biological characteristics make it suitable for experimental studies (e.g. small size, easy breeding, high fecundity; Miklosi & Andrew, 2006; Lawrence, 2007). Its short life cycle and rapid development also facilitate investigations regarding the effects of environmental factors on different lifestages. In the last two decades, some studies have been published regarding the metabolic performance of the *D. rerio* exposed to various environmental constraints (Plaut & Gordon, 1994; Plaut, 2000; Marit & Weber, 2011, 2012). Despite the increasing interest in *D. rerio*, only the studies of Barrionuevo & Burggren (1999) and Burggren (2005) investigated how body mass influenced metabolism, although they did not establish the regression describing this relationship. The aim of the present study was therefore to investigate the allometric relationship between body mass and metabolic rate in *D. rerio*, and derive the

species-specific scaling exponent. This was achieved by measuring  $R_A$  and  $R_S$  at three different life stages.

Ten broodstock couples (wild-type Tuebingen strain, *TU*) were reared together in a 10 l tank. Spawnings were obtained from these couples each week over two months. In order to avoid any bias due to familial characteristics, at least 5 spawns were mixed. Then, 100 larvae were randomly selected and reared to be tested at three different life stages: (i) larvae, 5 days post fertilization, (ii) 60 dpf juveniles and (iii) 5 months old adults. From 2 weeks onwards, fish were kept in groups of 10 individuals in 3 l aquaria with system water prepared as a mix of reverse-osmosis treated and tap water (both being filtered through sediment and activated charcoal filters) to obtain a water with constant conductivity of 300  $\mu$ S. Fish were reared at a constant temperature of 28 °C under artificial light with a constant photoperiod of 14h:10h (L:D) and were fed with dry food (Inicio plus, Biomar, France, <http://www.biomar.com>) and brine shrimp (Océan Nutrition Europe BVBA, Belgium, <http://www.oceannutrition.eu>) in the morning and afternoon, respectively. Fish were fasted 24 h prior to the experiments.

To assess their metabolic rate, fish were placed into circular size-adapted respirometers. For larvae, the set-up consisted of 4 independent glass micro respirometer chambers (diameter  $d = 1.12$  cm, volume  $V = 0.985 \cdot 10^{-3}$  l; Loligo systems, Denmark). For the two other life stages, 8 larger respirometers were run simultaneously ( $d = 3.75$  cm,  $V = 0.061$  l and  $7.50$  cm,  $0.179$  l for juveniles and adults respectively). Respirometers were submerged into buffer tanks (depth x length x height: 10x20x31 cm for larvae, 10x75x75 cm for juveniles and adults) and filled with temperature-controlled, oxygenated system water as above. Flush pumps controlled the water supply in each respirometer. Each flush pump was controlled by a timer, allowing intermittent flow respirometry (Steffensen, 1989) where phases of oxygen renewal alternated with phases of oxygen consumption measurements with a period of 15:15 min for larvae and

30:30 min for both juveniles and adults. In addition, the set-up was completed by a multi-channel peristaltic pump was used to mix the water within each respirometer. Each respirometer was equipped with a fiber optic sensor (PreSens) connected to a multichannel oxygen measuring system (OXY 4 mini, PreSens) to record dissolved oxygen levels. Optic fibers were calibrated at 0% and 100% of air saturation at a temperature of 28°C. A factor of conversion based on oxygen solubility into water was used to convert oxygen data from percentage saturation to  $\text{mgO}_2\cdot\text{l}^{-1}$  (i.e. 100% was equivalent to  $7.94\text{mgO}_2\cdot\text{l}^{-1}$  for a 28°C temperature and a 0 salinity). Oxygen saturation was recorded every five seconds with the program Oxyview (PreSens).

Larvae were tested in groups of 10 individuals per chamber. Juveniles and adults were tested individually; each fish was only tested once. Each experimental trial comprised two consecutive steps. First, to assess  $R_A$ , fish metabolism was increased through chasing (Schurmann & Steffensen, 1997; Lefrançois & Claireaux, 2003; Jourdan-Pineau *et al.*, 2010; Cannas *et al.*, 2013, Clark *et al.*, 2012). Each group of larvae or individual fish was transferred from the rearing aquaria to a 6-7 ml Petri dish or one liter tank respectively, where they were chased with a stick. When exhausted (i.e. did not respond to stimulation), they were transferred in respirometers to measure oxygen consumption over 20 min. After this first measurement of oxygen consumption, a second one was performed to confirm the accuracy of the  $R_A$  assessment. To do that fish were chased again inside the respirometer and their oxygen consumption measured for a new period of 20 min. To estimate  $R_S$ , fish were left undisturbed in the respirometer for 48h, during which oxygen consumption was regularly and automatically measured. After the 48h period of measurements, the fish were removed from the respirometers and anesthetized with benzocaine at a concentration of  $50\text{mg}\cdot\text{l}^{-1}$ . The wet body mass of each individual was determined, as well as the standard length  $L_S$  of juveniles and adults: (a) larvae, 5 dpf ( $n=11$  groups of 10 larvae;  $\text{mean} \pm \text{S.E.}$ , mass,  $M=0.245\cdot 10^{-3} \pm 0.036\cdot 10^{-3}$

<sup>3</sup>g), (b) 60 dpf juveniles ( $n=14$ ,  $M=0.097 \pm 0.035$ g,  $L_S=18.5 \pm 2.2$  mm) and (c) 5 months adults ( $n=22$ ,  $M=0.326 \pm 0.103$ g,  $L_S=26.9 \pm 1.9$  mm).

A blank measurement without fish was performed before and after each trial, to quantify background respiration. Linear change is assumed in background oxygen consumption over the 48h-experimental trial and subtracted the calculated background from the corresponding total oxygen consumption measured.

The non specific aerobic metabolic rate (or oxygen consumption  $MO_2$  in  $mgO_2.h^{-1}$ ) was calculated according to the following formula:

$$MO_2 = \Delta[O_2] \cdot V \cdot \Delta t^{-1} \quad \text{equation (1)}$$

where  $\Delta[O_2]$  (in  $mgO_2.l^{-1}$ ) is the variation in oxygen concentration during the measurement period  $\Delta t$  (in h) and  $V$  (in l) is the volume of the respirometer.  $MO_2$  correspond to the  $R_A$ , the maximal  $MO_2$  obtained after the fish being chased, or to the  $R_S$ , which is the  $MO_2$  assessed according to the method described by Steffensen et al. (1994). Briefly, to assess  $R_S$ , the frequency distribution of the  $MO_2$  values was plotted recorded during the last 24 hours of each trial. This generally produced a bimodal distribution where the higher and the lower mode were considered to reflect routine metabolic rate, i.e. energy required by the animal for normal activity, and  $R_S$ , respectively.  $R_A$  and  $R_S$  were assessed for each individual.

The non specific aerobic metabolic rate of organisms typically increases with body mass according to the allometric equation:

$$R = aM^b \quad \text{equation (2)}$$

where  $R$  is the metabolic rate ( $R_S$  or  $R_A$  in  $\text{mgO}_2\cdot\text{h}^{-1}$ ),  $a$  is the species-specific scaling constant (or proportionality constant),  $M$  is the body mass (in g), and  $b$  is the scaling exponent. This equation is a power function, where the value of  $b$  provides information on how the variable of interest changes with body size.

Equation (2) can be linearized with a log transformation:

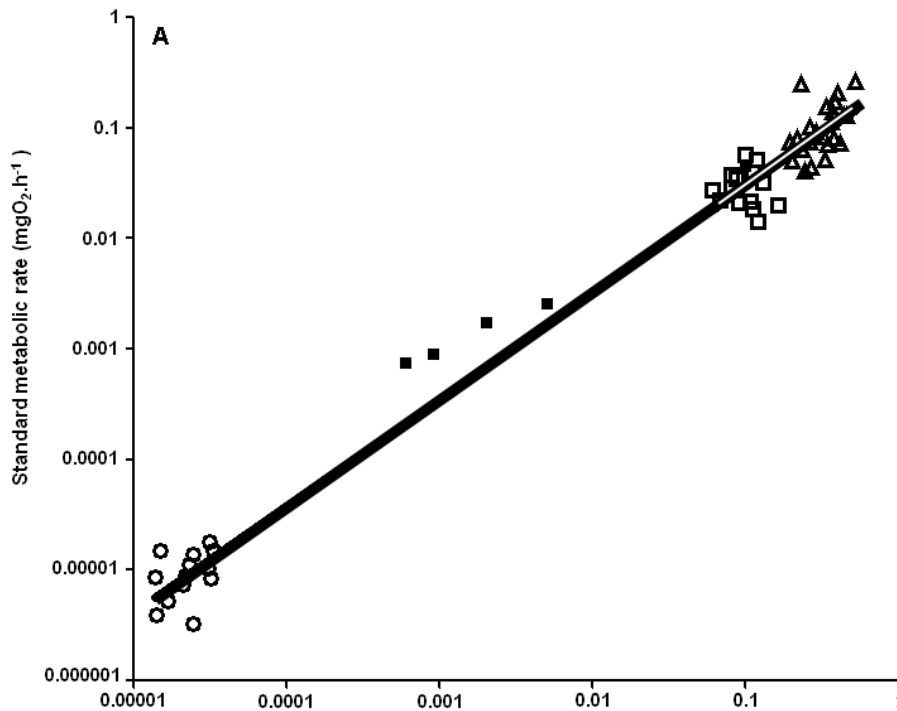
$$\log Y = \log a + b \log M \quad \text{equation (3)}$$

The logarithm base 10 of each metabolic variable ( $R_S$  or  $R_A$ ) was therefore plotted against the logarithm base 10 of body mass. Fitting a linear regression allowed provided the scaling exponent  $b$  and the log of the scaling coefficient ( $\log a$ ), i.e. the slope and the intercept, respectively. Previous studies in fishes (Post & Lee, 1996; Killen, 2007; Moran & Wells, 2007) have argued the metabolic rate of larvae is likely to scale differently from juveniles and adults because of their incomplete development. These groups were, therefore, analyzed separately. A first linear regression was therefore derived for the three stages and a second considering only juveniles and adults. The two scaling exponents  $b$  and scaling factors  $a$  were then compared as described by Zar (1984) with a modification of the t-test. Results were considered significant at  $P < 0.05$ .

For  $R_S$ , scaling factor  $a_{RS}$  and scaling exponent  $b_{RS}$  was  $0.273 \pm 0.038$  ( $\pm$  S.E.) and  $0.965 \pm 0.015$ , respectively when the three life stages were considered. These constants were  $0.276 \pm 0.084$  and  $0.969 \pm 0.110$ , respectively, when only juveniles and adults were taken into consideration. These values did not differ significantly for either constant (Student's t-test,  $t_{1.82} = -0.00127$ ,  $P > 0.05$ ; Student's t-test,  $t_{1.81} = 0.00068$ ,  $P > 0.05$ , respectively). The same pattern was observed for  $R_A$ . Scaling factors ( $a_{RA(L+J+A)} = 0.799 \pm 0.024$  and



173  $a_{RA(J+A)}=0.809\pm0.051$ ) as well as scaling exponents ( $b_{RA(L+J+A)}=0.926\pm0.009$  and  
 174  $b_{RA(J+A)}=0.931\pm0.068$ ) did not differ significantly whether larvae were considered or not  
 175 (Student's t-test, for  $a_{RA}$ :  $t_{1.81}=0.01270$ ,  $P>0.05$ ; for  $b_{RA}$ :  $t_{1.81}=0.00596$ ,  $P>0.05$ ). Data for  
 176 "resting metabolic rate" of *D. rerio* at 28°C, reported by Barrionuevo & Burggren (1999) and  
 177 Burggren (2005) and converted to  $\text{mgO}_2\cdot\text{h}^{-1}$ , were consistent with  $R_S$  values measured in the  
 178 current study (Fig. 1A). It appears that their data fit with standard metabolic rate regression.  
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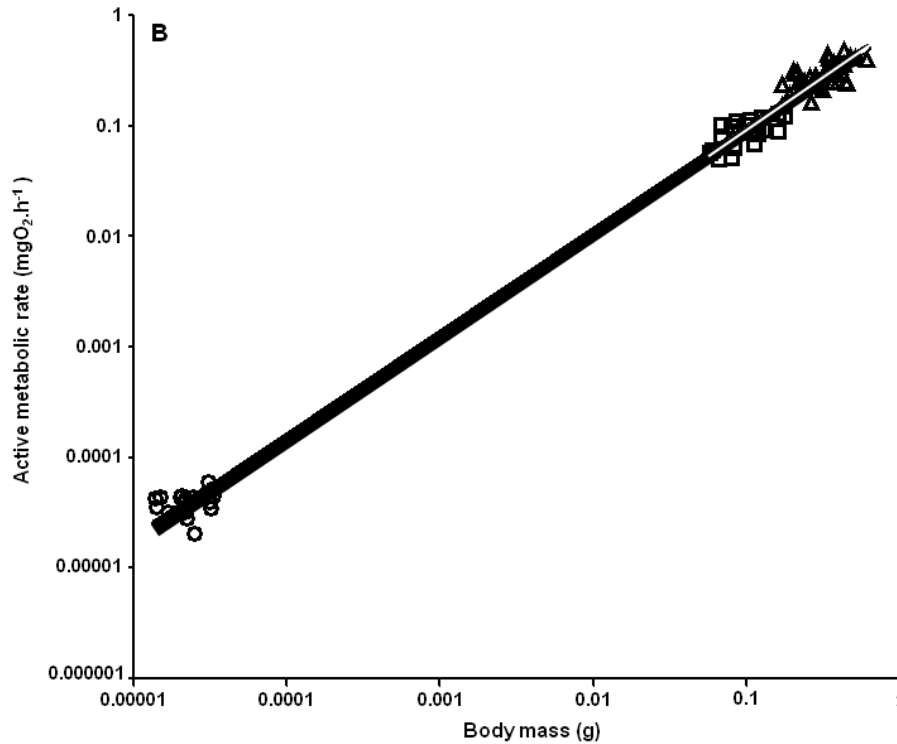


FIG. 1. Relationships between aerobic metabolic rate and body mass for *D. rerio*. On each graph, the black line represents the scaling relationship across the three life stages: 5 day-old larvae ( $\circ$ , L), 2 month-old juveniles ( $\square$ , J) and 6 month-old adults ( $\Delta$ , A). The white line shows the scaling only for juveniles and adults (J+A). The scaling factor  $a$  and scaling exponent  $b$  were estimated through the allometric equation  $R=aM^b$  (equation 2). For each of these constants, the value is expressed as the average ( $\pm$  standard error). For standard metabolic rate (graph A), considering all stages  $R_{S(L+J+A)}$  is equal to  $0.799 (\pm 0.038) M^{0.965 (\pm 0.085)}$  for  $n=50$  ( $P<0.0001$ ), considering juveniles and adults,  $R_{A(J+A)}$  is equal to  $0.809 (\pm 0.084) M^{0.969 (\pm 0.110)}$  for  $n=37$  ( $P<0.0001$ ). Regarding active metabolic rate (graph B):  $R_{A(L+J+A)}$   $0.273(\pm 0.024)M^{0.926(\pm 0.010)}$  for  $n=78$  ( $P<0.0001$ ),  $R_{A(J+A)}$   $0.276(\pm 0.051)M^{0.931(\pm 0.068)}$  for  $n=60$  ( $P<0.0001$ ). In addition, the studies of Barrionuevo & Burggren (1999) and Burggren (2005) measured oxygen consumption in fish presenting a resting state at the temperature of  $28^\circ\text{C}$  which permitted to complete our data set regarding the standard metabolic rate at the same temperature. After conversion into  $\text{mgO}_2\cdot\text{h}^{-1}$ , these values were added on the graph (A,  $\blacksquare$ ).

This is the first study examining the scaling of metabolic rate with body mass over the lifecycle of *D. rerio*. The results indicate that a single scaling exponent can be employed irrespective of life stages in this species. This is in agreement with the observations made by Killen *et al.* (2007) on three species of marine teleost (e.g. ocean pout *Macrozoarces americanus* Bloch & Schneider 1801; lumpfish *Cyclopterus lumpus*, L. 1758; and shorthorn sculpin *Myoxocephalus scorpius*, L. 1758), while the contrary was shown by

Post & Lee (1996) for other species (e.g. the common carp *Cyprinus carpi*, L. 1758; rainbow trout *Oncorhynchus mykiss*, Walbaum 1792; sea bream *Pagrus major*, Temminck & Schlegel 1843). White & Seymour (2011) studied mass-specific  $R_S$  of 31 fish species and reported that several species have higher allometric exponents in early larval stages compared to their later stages. Also, in their study of yellowtail kingfish *Seriola lalandi* (Valenciennes 1833), Moran & Wells (2007) considered that the allometric exponent changed continually during development. In fact, metabolism in larvae may be affected by processes specific to this early ontogenic stage such as higher rates of protein turnover, development of energy consuming tissue or organs, or the progressive transition from cutaneous gas exchange to branchial respiration or changes in swimming ability (Post & Lee, 1996; Killen *et al.*, 2007; White & Seymour, 2011; Gore & Burggren, 2012). Nonetheless, understanding of ontogenic changes in aerobic scope in fishes throughout remains limited. In *D. rerio*, the general pattern observed may be related to its short lifecycle, 5dpf larvae having already completed most of their organogenesis (Kimmel *et al.*, 1995). It could be interesting to obtain data for 21dpf, when larval metamorphosis occurs with maturation of several physiological functions and the completion of the transfer of respiratory gas exchange from predominantly cutaneous to predominantly branchial.

As expected, the scaling exponents  $b_{RA}$  and  $b_{RS}$  differed significantly from 1, confirming that metabolic rate scaled allometrically with body mass in this species (Student's t-test,  $t_{1.47}=55.88$ ,  $P<0.0001$  for  $b_{RA}$ ;  $t_{1.47}=5.224$ ,  $P<0.05$  for  $b_{RS}$ ).

Although studies by Kleiber (1932) on mammals and Hemmingsen (1960) on unicells, multicellular ectotherms and endotherms suggested that 0.75 was a universal scaling exponent, recent investigations on fishes have revealed significant heterogeneity in scaling exponents among species (Glazier, 2005, 2006, 2009c; White *et al.*, 2006; Downs *et al.*, 2008; White, 2011). Among the teleosts, several studies have found that the scaling exponent of

metabolic rate differed from 0.75 (Post & Lee 1996; Clark & Johnston, 1999; Bokma, 2004; Peck, 2004), which is in agreement with the present results. It is worth noticing that a lot of scaling studies in teleosts are based on routine or “resting” metabolic rate, rather than  $R_S$  or  $R_A$ . This is not directly comparable with the present results, but illustrates the interspecific variation in allometric scaling exponents among fishes. For instance, Moran & Wells (2007) found a scaling exponent of 0.90 for *S. lalandi*. Bokma (2004) examined intraspecific allometry for the sea trout *Salmo trutta* in various life stages and found  $b_{RMR}$  to be 0.86. Indeed, lifestyle, swimming mode and environmental characteristics of the habitat are all known to modify metabolic rate, as well as the scaling exponent in fishes (Killen *et al.*, 2007, 2010). Previous studies have also found that  $b_{RA}$  was significantly different from  $b_{RS}$  (Brett & Glass, 1973; Weiser, 1985; Weibe *et al.*, 2004; Killen *et al.*, 2007), and this was true in *D. rerio* (Student’s t-test  $t_{1,94}=4.475$ ,  $P<0.05$ ), which is also suggested by several studies. However,  $b_{RA}$  generally tends to be higher than  $b_{RS}$  (Weibe *et al.*, 2004; Killen *et al.*, 2007) while the opposite was observed in *D. rerio*, where  $b_{RA}<b_{RS}$ . This may, at least in part, reflect the fact that *D. rerio* is a domesticated species that has been reared in laboratory for many generations, whereas the study by Killen *et al.* (2007) was on individuals captured in wild. In conclusion, this study estimated the scaling exponent  $b$  across three life stages of *D. rerio* (5 dpf to 6 month old fish). This provides a basis for further experiments regarding the effects of environmental factors on aerobic metabolism in this species.

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