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Allometric relationship between body mass and aerobic metabolism in zebrafish Danio rerio

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Abstract :

The relationship between body mass (M) and metabolic rate was investigated through the assessment of active (RA) and standard (RS) metabolic rate at different life stages in zebrafish Danio rerio (5 day-old larvae, 2month-old juveniles and 6 month-old adults). Scaling exponents and constants were assessed for standard (RS =0·273M0·965 in mgO2 g−1 h−1) and active metabolic rate (RA =0·799M0·926 in mgO2 g−1 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.

Keywords : active metabolic rate, static respirometry, allometric scaling exponent, standard metabolic rate

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) isthe 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 andsalinity (Shurmann&Steffensen, 1997; Lefrançois&Claireaux, 2003; Johansen & Jones, 2011). Ithas therefore been used in many studies to assess the influence of environmental factors on metabolic performance and energy-demanding activitiesin various fish species (Fry, 1971; Priede, 1985; Claireaux&Lefrançois, 2007).It has been demonstrated, in many species however, thatintrinsic 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 publishedregarding the metabolic performance ofthe *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 allometricrelationship between body mass and metabolic rate in *D.rerio*, and derive the

73 species-specific scaling exponent. This was achieved by measuring R_A and $R_S$atthree different
74 life stages.
75
76 Tenbroodstock couples (wild-type Tuebingenstrain, *TU*) werereared together in a 10 l tank.
77 Spawnings were obtained from these couples each week over two month. In order to avoid
78 any bias due to familial characteristics, at least 5 spawns were mixed. Then, 100 larvae were
79 randomly selected and reared to be tested at three different life stages: (i) larvae, 5 days post
80 fertilization, (ii) 60 dpf juveniles and (iii) 5 months old adults. From 2 weeks onwards, fish
81 were kept in groups of 10 individuals in 3l aquaria with system water prepared as a mix of
82 reverse-osmosis treated and tap water (both being filtered through sediment and activated
83 charcoal filters) to obtain a water with constant conductivity of 300µS. Fish were reared at a
84 constant temperature of 28 °C under artificial light with a constant photoperiod of 14h:10h
85 (L:D) and were fed with dry food (Inicio plus, Biomar, France, http://www.biomar.com) and
86 brine shrimp (Océan Nutrition Europe BVBA, Belgium, http://www.oceannutrition.eu) in the
87 morning and afternoon, respectively. Fish were fasted 24 h prior to the experiments.
88 To assess their metabolic rate, fish were placed into circular size-adapted respirometers. For
89 larvae, the set-up consisted of 4independent glass micro respirometer chambers (diameter d=
90 1.12 cm, volume V= $0.985.10^{-3}$l; Loligo systems, Denmark). For the two other life stages, 8
91 larger respirometers were run simultaneously (d= 3.75cm, V=0.061l and 7.50 cm, 0.179 l for
92 juveniles and adults respectively).Respirometers were submerged into buffer tanks (depth x
93 length x height: 10x20x31cm for larvae, 10x75x75xcmfor juveniles and adults) and filled
94 with temperature-controlled, oxygenated system water as above. Flush pumps controlled the
95 water supply in each respirometer. Each flush pump was controlled by a timer, allowing
96 intermittent flow respirometry (Steffensen, 1989) where phases of oxygen renewal alternated
97 with phases of oxygen consumption measurements with a period of 15:15min for larvae and

30:30 min for both juvenilesand adults. In addition, the set-up was completed by amulti-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 $mgO_2.l^{-1}$ (i.e. 100% was equivalent to $7.94mgO_2.l^{-1}$ 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 assessR_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 *RA* assessment. To do that fish were chased again inside the respirometer and their oxygen consumptionmeasured for a new period of 20 min.To estimateR_S, fish were left undisturbed in the respirometerfor 48h, during which oxygen consumptionwas regularly and automatically measured. After the 48h period of measurements, the fish were removed from the respirometers and anesthetized with benzocaïne at a concentration of $50mg.l^{-1}$. 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; mean±S.E., mass, $M=0.245.10^{-3}\pm0.036.10^{-}$

^{3}g), (b) 60 dpf juveniles (n=14, M=0.097 ± 0.035g, L_S=18.5 ± 2.2 mm) and (c) 5 months adults (n=22, M=0.326 ± 0.103g, L_S=26.9 ± 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 consumptionover the 48h-experimental trial and subtracted the calculated background from the corresponding total oxygen consumptionmeasured.

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

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

where$\Delta[O_2]$ (in $mgO_2.l^{-1}$) is the variation in oxygen concentration during the measurement periodΔt(in h) and V(in l) is the volume of the respirometer. MO_2correspond to the R_A,themaximalMO_2obtained after the fish being chased, or to the R_S, which is the MO_2assessed according to the method described by Steffensen et al. (1994). Briefly, to assess R_S, thefrequency distribution of the MO_2values 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:

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

where*R* is the metabolic rate (R_S or R_A in $mgO_2.h^{-1}$), 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.

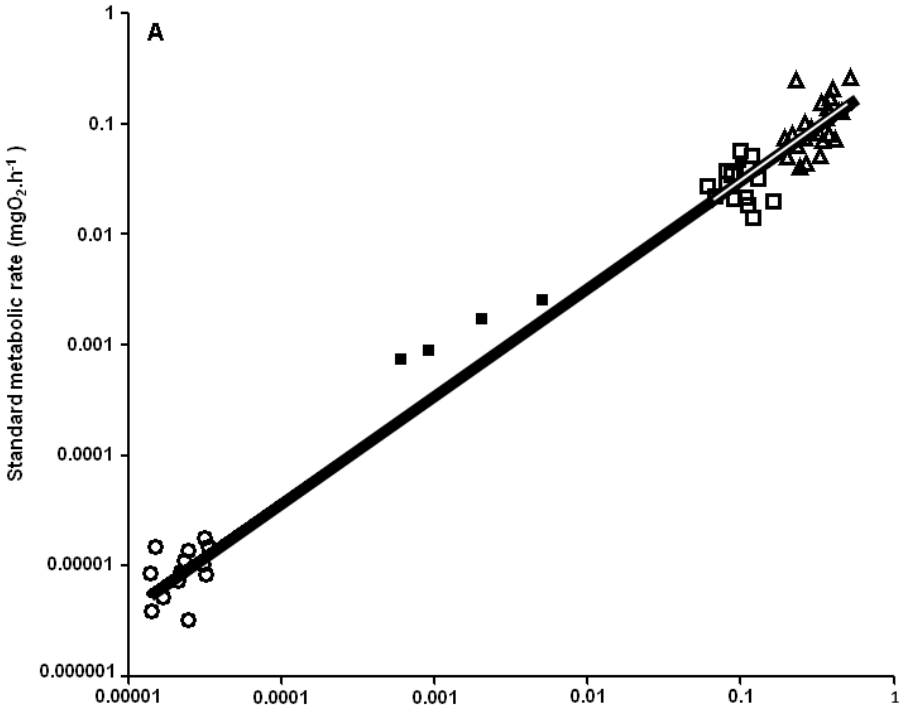
Equation (2) can be linearized with a log transformation:

$$\log Y = \log a + b \log M \qquad \textit{equation (3)}$$

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 (loga), i.e. the slope and the intercept, respectively. Previous studies in fishes (Post & Lee, 1996; Killen, 2007; Moran & Wells, 2007) have argued themetabolic 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 werethen 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±0.038 (± S.E.) and 0.965±0.015, respectively when the three life stages were considered. These constantswere 0.276±0.084 and 0.969±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±0.024 and

173 $a_{RA(J+A)}$=0.809±0.051) as well as scaling exponents ($b_{RA(L+J+A)}$=0.926±0.009 and
174 $b_{RA(J+A)}$=0.931±0.068)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 byBarrionuevo&Burggren (1999) and
177 Burggren (2005) and converted to $mgO_2.h^{-1}$, 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.
179



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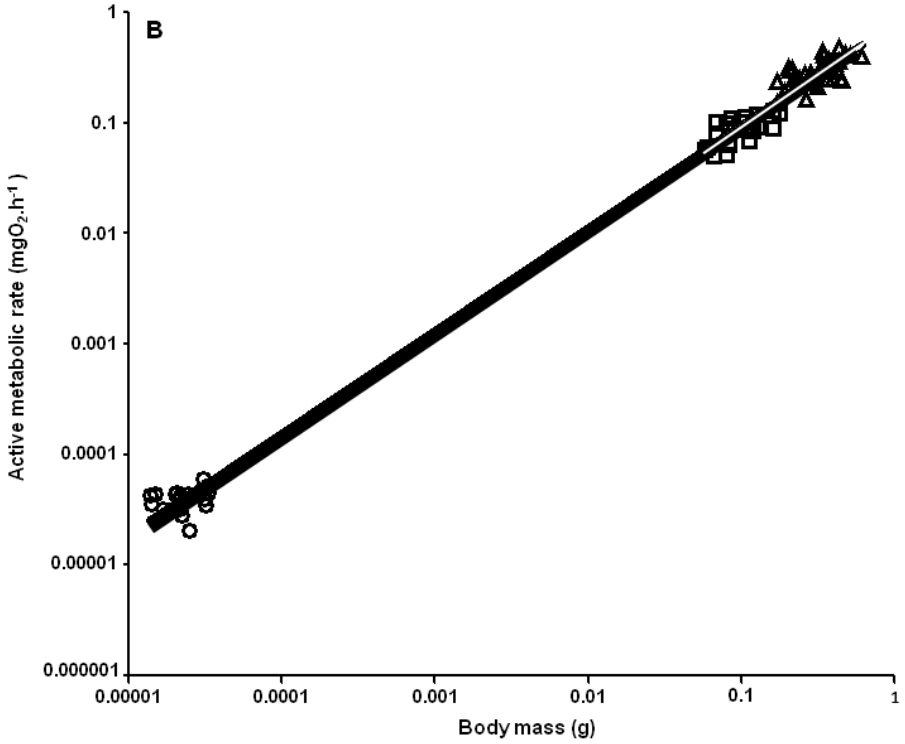

FIG. 1. Relationships between aerobic metabolic rate and body mass for D. rerio. On each graph, the black line represents the scaling relationship across the three life stages: 5 day-old larvae (○, L), 2 month-old juveniles (□, J) and 6 month-old adults (Δ, A). The white line shows the scaling only for 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 (± standard error). For standard metabolic rate (graph A), considering all stages $R_{S(L+J+A)}$ is equal to 0.799 (±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 (±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 °C which permitted to complete our data set regarding the standard metabolic rate at the same temperature. After conversion into $mgO_2.h^{-1}$, these values were added on the graph (A, ■).

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 stgae 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*Macrozoarcesamericanus*Bloch & Schneider 1801; lumpfish *Cyclopteruslumpu,s*L. 1758; and shorthorn sculpin*Myoxocephalusscorpius,* 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 *Oncorhynchusmykis*, Walbaum 1792; sea bream *Pagrusmajo*, Temmink& 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 *Seriolalalandi* (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).Nontheless, 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) onunicells, 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 *Salmotrutatruta* 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; Weibel*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}generallytends to be higher than b_{RS}(Weibel*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 metabolismin this species.

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