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24 The relationship between body mass and metabolic rate was investigated through the

25 assessment of active and standard metabolic rate at different life stages inzebrafish*Daniorerio*

26 (5 day-old larvae, 2 month-old juveniles and 6 month-old adults). Scaling exponents and

27 constants were assessed for standard ($R_S=0.273M^{0.965}$ in $mgO^2.g^{-1}.h^{-1}$) and active metabolic

28 rate ($R_A=0.799M^{0.926}$ in $mgO^2.g^{-1}.h^{-1}$). These data provide the basis for further experiments

29 regarding the effects of environmental factors on aerobic metabolism throughout the life cycle

30 of this species.

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32 Key words: allometric scaling exponent, aerobic metabolism, zebrafish

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48   Active metabolic rate (R_A) is the maximal aerobic metabolic rate of an organism in a highly

49   active state and standard metabolic rate (R_S) isthe minimal metabolic rate necessary for

50   supporting maintenance activities (e.g. ventilation) measured in resting, starved and non-

51   maturing individual (Fry, 1947; Brett, 1964). Aerobic metabolic scope (R_{AS}) is the difference

52   between R_A and R_S and represents the capacity of an organism to provide oxygen to sustain

53   energy demanding activities (Fry, 1947). R_{AS}can be influenced by a set of environmental

54   parameters such as temperature, oxygen andsalinity (Shurmann&Steffensen, 1997;

55   Lefrançois&Claireaux, 2003; Johansen & Jones, 2011). Ithas therefore been used in many

56   studies to assess the influence of environmental factors on metabolic performance and energy-

57   demanding activitiesin various fish species (Fry, 1971; Priede, 1985; Claireaux&Lefrançois,

58   2007).It has been demonstrated, in many species however, thatintrinsic parameters such as

59   ontogeny and body size can also influence metabolism independently of any effects of

60   environmental conditions (Clark & Johnston, 1999; Bokma, 2004;Killen, 2007, 2010; Moran

61   & Wells, 2007)

62   Zebrafish*Daniorerio*(Hamilton 1822) is a small teleost species whose biological

63   characteristics make it suitable for experimental studies (*e.g.* small size, easy breeding, high

64   fecundity; Miklosi& Andrew, 2006; Lawrence, 2007). Its short life cycle and rapid

65   development also facilitate investigations regarding the effects of environmental factors on

66   different lifestages. In the last two decades,some studies have been publishedregarding the

67   metabolic performance ofthe *D.rerio*exposed to various environmental constraints (Plaut&

68   Gordon, 1994; Plaut, 2000; Marit& Weber, 2011, 2012). Despite the increasing interest in

69   *D.rerio*, only the studies of Barrionuevo&Burggren (1999) and Burggren (2005) investigated

70   how body mass influenced metabolism, although they did not establish the regression

71   describing this relationship.The aim of the present study was therefore to investigate the

72   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 ($d= 3.75$cm, $V=0.061$l 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

98   30:30 min for both juvenilesand adults. In addition, the set-up was completed by amulti-

99   channel peristaltic pump was used to mix the water within each respirometer. Each

100 respirometer was equipped with a fiber optic sensor (PreSens) connected to a multichannel

101 oxygen measuring system (OXY 4 mini, PreSens)to record dissolved oxygen levels. Optic

102 fibers were calibrated at 0% and 100% of air saturation at a temperature of 28°C. A factor of

103 conversion based on oxygen solubility into water was used to convert oxygen data from

104 percentage saturation to $mgO_2.l^{-1}$ (i.e. 100% was equivalent to $7.94 mgO_2.l^{-1}$ for a 28°C

105 temperature and a 0 salinity).Oxygen saturation was recorded every five seconds with the

106 program Oxyview (PreSens).

107 Larvae were tested in groups of 10 individuals per chamber. Juveniles and adults were tested

108 individually; each fish was only tested once. Each experimental trial comprised two

109 consecutive steps. First,to assessR_A, fish metabolism was increased through chasing

110 (Schurmann&Steffensen, 1997; Lefrançois&Claireaux, 2003; Jourdan-Pineau$et\ al.$, 2010;

111 Cannas $et\ al.$, 2013, Clark $et\ al.$, 2012). Each group of larvae or individual fish was

112 transferred from the rearing aquaria to a 6-7 ml Petri dish or one liter tank respectively, where

113 they were chased with a stick. When exhausted (i.e. did not respond to stimulation), they were

114 transferred in respirometers to measure oxygen consumption over 20 min. After this first

115 measurement of oxygen consumption, a second one was performed to confirm the accuracy of

116 the RA assessment. To do that fish were chased again inside the respirometer and their oxygen

117 consumptionmeasured for a new period of 20 min.To estimateR_S, fish were left undisturbed in

118 the respirometerfor 48h, during which oxygen consumptionwas regularly and automatically

119 measured. After the 48h period of measurements, the fish were removed from the

120 respirometers and anesthetized with benzocaïne at a concentration of $50 mg.l^{-1}$. The wet body

121 mass of each individual was determined, as well as the standard length L_S of juveniles and

122 adults: (a) larvae, 5 dpf (n=11 groups of 10 larvae; mean±S.E., mass, M=0.245.10^{-3}±0.036.10^{-}

123 ^{3}g), (b) 60 dpf juveniles (n=14, M=0.097 ± 0.035g, L_S=18.5 ± 2.2 mm) and (c) 5 months

124 adults (n=22, M=0.326 ± 0.103g, L_S=26.9 ± 1.9 mm).

125

126 A blank measurement without fish was performed before and after each trial, to quantify

127 background respiration.Linear change is assumed in background oxygen consumptionover the

128 48h-experimental trial and subtracted the calculated background from the corresponding total

129 oxygen consumptionmeasured.

130 The non specific aerobic metabolic rate (or oxygen consumption MO_2 in $mgO_2.h.^{1}$) was

131 calculated according to the following formula:

132

133 $$MO_2 = \Delta[O_2].V. \Delta t^{-1} \quad \textit{equation (1)}$$

134

135 where$\Delta[O_2]$ (in $mgO_2.l^{-1}$) is the variation in oxygen concentration during the measurement

136 periodΔt(in h) and V(in l) is the volume of the respirometer. MO_2correspond to the

137 R_A,themaximalMO_2obtained after the fish being chased, or to the R_S, which is the

138 MO_2assessed according to the method described by Steffensen et al. (1994). Briefly, to assess

139 R_S, thefrequency distribution of the MO_2values was plotted recorded during the last 24 hours

140 of each trial. This generally produced a bimodal distribution where the higher and the lower

141 mode were considered to reflect routine metabolic rate, i.e. energy required by the animal for

142 normal activity, and R_S, respectively.R_A and R_S were assessed for each individual.

143 The non specific aerobic metabolic rate of organisms typically increases with body mass

144 according to the allometric equation:

145

146 $$R = aM^b \textit{equation (2)}$$

147

148 where$R$ is the metabolic rate (R_S or R_A in $mgO_2.h^{-1}$), a is the species-specific scaling constant

149 (or proportionality constant), M is the body mass (in g), and b is the scaling exponent. This

150 equation is a power function, where the value of b provides information on how the variable

151 of interest changes with body size.

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

153

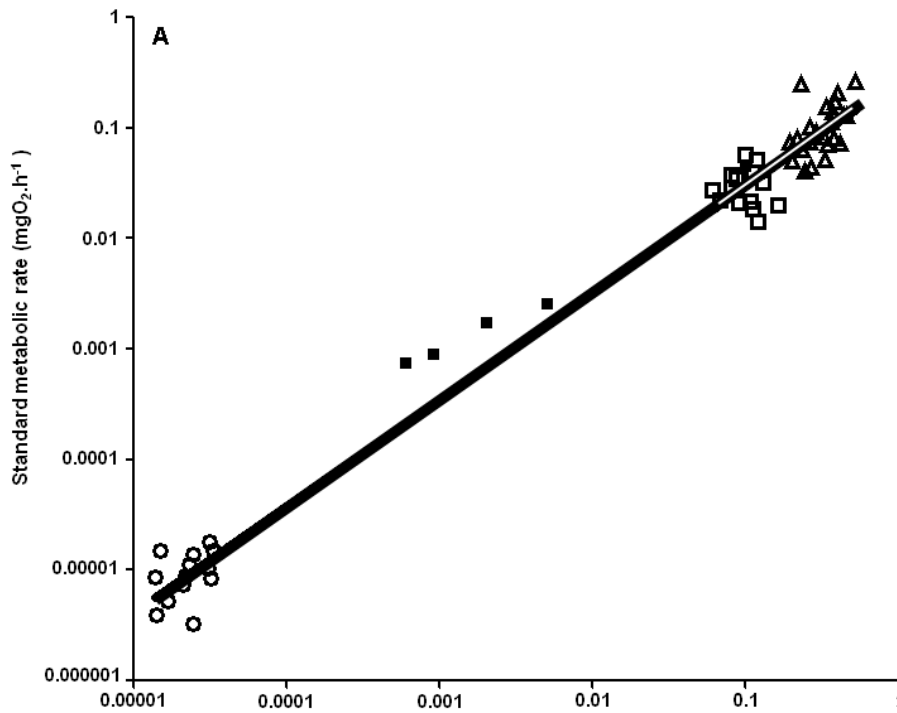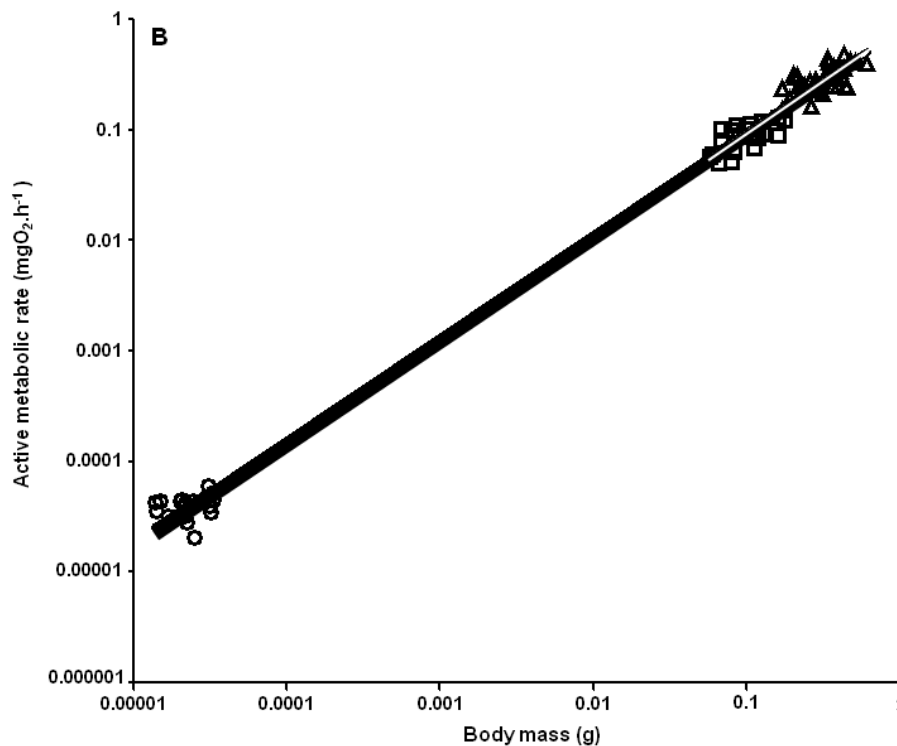154 $$\log Y = \log a + b \log M \qquad equation\ (3)$$

155

156 The logarithm base 10 of each metabolic variable (R_S or R_A)was therefore plotted against the

157 logarithm base 10 of body mass. Fitting a linear regression allowed provided the scaling

158 exponent b and the log of the scaling coefficient (loga), i.e. the slope and the intercept,

159 respectively. Previous studies in fishes (Post & Lee, 1996; Killen, 2007; Moran & Wells,

160 2007) have argued themetabolic rate of larvae is likely to scale differently from juveniles and

161 adults because of their incomplete development. These groups were, therefore, analyzed

162 separately. A first linear regression was therefore derived for the three stages and a second

163 considering only juveniles and adults. The two scaling exponents b and scaling factors a

164 werethen compared as described by Zar (1984) with a modification of the t-test. Results were

165 considered significant at$P<0.05$.

166

167 For R_S, scaling factor a_{RS} and scaling exponent b_{RS} was 0.273 ± 0.038 ($\pm$ S.E.) and

168 0.965 ± 0.015, respectively when the three life stages were considered. These constantswere

169 0.276 ± 0.084 and 0.969 ± 0.110, respectively, when only juveniles and adults were taken into

170 consideration. These values did not differ significantly for either constant(Student's t-test,

171 $t_{1.82}=-0.00127$, $P>0.05$; Student's t-test, $t_{1.81}=0.00068$, $P>0.05$, respectively). The same

172 pattern was observed for R_A. Scaling factors ($a_{RA(L+J+A)}=0.799\pm0.024$ 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 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 $0.799\ (\pm0.038)\ M^{0.965\ (\pm0.085)}$ for n=50 (P<0.0001), considering juveniles and adults, $R_{A(J+A)}$ is equal to $0.809\ (\pm0.084)\ M^{0.969\ (\pm0.110)}$ for n=37 (P<0.0001). Regarding active metabolic rate (graph B): $R_{A(L+J+A)}\ 0.273(\pm0.024)M^{0.926(\pm0.010)}$ for n=78 (P<0.0001), $R_{A(J+A)}$ $0.276(\pm0.051)M^{0.931(\pm0.068)}$ 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

202   Post & Lee (1996) for other species (e.g. the common carp *Cyprinus carpi,* L. 1758; rainbow

203   trout *Oncorhynchusmykis,* Walbaum 1792; sea bream *Pagrusmajo,* Temmink& Schlegel

204   1843). White & Seymour (2011) studied mass-specific R_S of 31 fish species and reported that

205   several species have higher allometric exponents in early larval stages compared to their later

206   stages. Also, in their study of yellowtail kingfish *Seriolalalandi* (Valenciennes 1833), Moran

207   & Wells (2007) considered that the allometric exponent changed continually during

208   development. In fact, metabolism in larvae may be affected by processes specific to this early

209   ontogenic stage such as higher rates of protein turnover, development of energy consuming

210   tissue or organs, or the progressive transition from cutaneous gas exchange to branchial

211   respiration or changes in swimming ability (Post & Lee, 1996; Killen*et al.*, 2007; White &

212   Seymour, 2011; Gore &Burggren, 2012).Nontheless, understanding of ontogenic changes in

213   aerobic scope in fishes throughout remains limited. In *D. rerio*, the general pattern observed

214   may be related to its short lifecycle, 5dpf larvae having already completed most of their

215   organogenesis (Kimmel *et al.*, 1995). It could be interesting to obtain data for 21dpf, when

216   larval metamorphosis occurs with maturation of several physiological functions and the

217   completion of the transfer of respiratory gas exchange from predominantly cutaneous to

218   predominantly branchial.

219   As expected, the scaling exponents b_{RA} and b_{RS} differed significantly from 1, confirming that

220   metabolic rate scaled allometrically with body mass in this species (Student's t-test,

221    $t_{1.47}=55.88$, P<0.0001 for b_{RA}; $t_{1.47}=5.224$, P<0.05 for b_{RS}).

222   Although studies by Kleiber (1932) on mammals and Hemmingsen (1960) onunicells,

223   multicellular ectotherms and endotherms suggested that 0.75 was a universal scaling

224   exponent, recent investigations on fishes have revealed significant heterogeneity in scaling

225   exponents among species (Glazier, 2005, 2006, 2009c; White *et al.*, 2006; Downs *et al.*, 2008;

226   White, 2011).Among the teleosts, several studies have found that the scaling exponent of

227 metabolic rate differed from 0.75 (Post & Lee 1996; Clark & Johnston, 1999; Bokma, 2004;

228 Peck, 2004), which is in agreement with the present results. It is worth noticing that a lot of

229 scaling studies in teleosts are based on routine or "resting" metabolic rate, rather than R_S or

230 R_A. This is not directly comparable with the present results, but illustrates the interspecific

231 variation in allometric scaling exponents among fishes. For instance, Moran & Wells (2007)

232 found a scaling exponent of 0.90 for *S. lalandi*. Bokma (2004) examined intraspecific

233 allometry for the sea trout *Salmotrutatruta* in various life stages and found b_{RMR} to be 0.86.

234 Indeed, lifestyle, swimming mode and environmental characteristics of the habitat are all

235 known to modify metabolic rate, as well as the scaling exponent in fishes (Killen *et al.*, 2007,

236 2010). Previous studies have also found that b_{RA} was significantly different from b_{RS} (Brett &

237 Glass, 1973; Weiser, 1985; Weibel*et al.*, 2004; Killen *et al.*, 2007), and this was true in *D.*

238 *rerio*(Student's t-test $t_{1.94}$=4.475, P<0.05), which is also suggested by several studies.

239 However, b_{RA}generallytends to be higher than b_{RS}(Weibel*et al*., 2004; Killen*et al*., 2007)

240 while the opposite was observed in *D. rerio,* where $b_{RA}<b_{RS}$. This may, at least in part, reflect

241 the fact that *D. rerio* is a domesticated species that has been reared in laboratory for many

242 generations, whereas the study by Killen*et al.* (2007) was on individuals captured in wild.

243 In conclusion, this study estimated the scaling exponent b across three life stages of *D. rerio*

244 (5 dpf to 6 month old fish). This provides a basis for further experiments regarding the effects

245 of environmental factors on aerobic metabolismin this species.

246

251 Poitou-Charentes. This study was conducted under the approval of Animal Care Committee of

252 France under the official licence of Marie-Laure Bégout (17-010).

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