Journal of Thermal Biology
February 2013, Volume 38, Issue 2, Pages 98–106
http://dx.doi.org/10.1016/j.jtherbio.2012.12.001

Trade-off between thermal sensitivity, hypoxia tolerance and growth in fish

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Abstract:

One outcome of contemporary climate trends is that the involvement of hypoxia and heat tolerance in determining individual fitness will increase in many fish populations. Large fish are believed to be more tolerant to hypoxia than small fish (Nilsson and Östlund-Nilsson, 2008) whereas thermal sensitivity is thought to decrease with body size (Clark et al., 2008). To better understand the bases of inter-individual variation in environmental adaptation performance, the current study examined hypoxia and heat tolerance in a fast growing (FGS; 288.3 ±14.4 g, 26.04±0.49 cm) and a slow growing (SGS; 119.95±6.41 g; 20.98±0.41 cm) strain of 1-year old rainbow trout (*Oncorhynchus mykiss*). This examination was conducted using two standardized challenge tests aimed at assessing individual incipient lethal oxygen saturation and incipient upper lethal temperature. Results to these tests were then cross-correlated with swim tests during which individual basal and active metabolic rate values were also measured. Measurements of permeabilized ventricular myofibers oxygen consumption were also conducted, as well as various organ-to-body-mass ratios. Experimental data showed that FGS was more hypoxia tolerant than SGS (13.4 to 16.7% air sat versus 14.7 to 18.9% air sat respectively). On the other hand, FGS was found less tolerant to heat than SGS (24.7–27.6 °C versus 28.5 to 29.7 °C respectively). Adding to the body size effect, another source of inter-individual variation in environmental tolerance was found. Residual analysis highlighted that whereas none of the individual morphometric and energetic traits correlated with hypoxia tolerance, permeabilized ventricular myofibers maximal oxygen consumption correlated well with individual tolerance to heat.

Highlights

► Fast growing fish are more hypoxia tolerant than slow growing fish. ► Fast growing fish are less tolerant to heat than slow growing fish. ► Permeabilized ventricular myofibers maximal oxygen consumption correlated with tolerance to heat.

Keywords: Fish ; Hypoxia ; Temperature ; Trade-off ; Growth ; Body size

INTRODUCTION

Since the beginning of the twentieth century, human population has been growing at an unprecedented rate (Keyfitz, 1989), causing serious damage to the earth ecosystem and altering global climate (Schneider, 1989). In the mean time, human activities have concentrated along riverbanks and marine coastlines, making these shallow aquatic ecosystems particularly exposed to anthropogenic influences and eutrophication. As a result, a combination of increased temperature and reduced oxygen availability is currently impinging upon several freshwater and marine fish populations (Ficke et al., 2007; Rabalais et al., 2009). In that context, prospects are that the involvement of hypoxia and heat tolerance in determining individual fitness is likely to increase in many fish populations. Unfortunately, predictions about how this may affect their production, dynamics and evolution are largely uncertain. This uncertainty partly result from the extensive intra-specific variation in environmental adaptation ability that is classically observed in fish (Pörtner et al., 2006; Claireaux and Lefrançois, 2007).

Body size is a potential source of inter-individual variability in environmental adaptation performance. Large fish are indeed believed to be more tolerant to hypoxia than small fish (Nilsson and Nilsson, 2008) whereas thermal sensitivity is thought to decrease with body size (Clark et al., 2008). However, information about whether a trade-off exists between these traits and how they may scale with body mass is scarce.

Thus, to better understand the bases of inter-individual variation in environmental adaptation performance, the present study examined some morpho-functional determinants of hypoxia and heat tolerance in fish. This examination was conducted using a methodology based on two standardized challenge tests aimed at assessing individual incipient lethal oxygen saturation (ILOS) and incipient upper lethal temperature (IULT). Results to these tests were then cross-correlated with swim tests during which individual critical swimming speed and standard and active metabolic rates were measured. Measurements of permeabilized

ventricular myofibers oxygen consumption were also conducted, as well as various organ-to-body-mass ratios. Two strains of rainbow trout (*Oncorhynchus mykiss*) were used for this experiment, a fast growing strain and a slow growing strain. Inter-strain comparison aimed at examining the influence of body size and growth on environmental adaptation performance, whereas intra-strain contrasting allowed the deciphering of size-independent sources of inter-individual variation. Moreover, the relevance of elements of scaling in understanding trade-offs between hypoxia tolerance and heat tolerance was examined.

MATERIALS AND METHODS

Fish

Rainbow trout (*Oncorhynchus mykiss*) issued from two different genetic strains, a fast growing strain (FGS; hatching November 15th 2009) and a slow growing strain (SGS; hatching January 12th 2010), were obtained from PEIMA experimental fish farm (Institut National de la Recherche Agronomique, Sizun, France). At the time of the experiments (January to March 2011) fish were approximately one year old and their mean mass and length (± SEM) were 288.3 ±14.4 g and 26.04 ±0.49 cm respectively for FGS (n = 20) and 119.95 ±6.41 g and 20.98 ±0.41 cm respectively for SGS (n = 19) (Table 1). Strains were transported to Université de Bretagne Occidentale rearing facility and placed in two, 500 L rearing tanks situated side by side in the same room. These tanks were supplied with the same aerated, biofiltered, sterilized (UV) and thermoregulated (12 °C), recirculating freshwater (renewal rate: 30 % per week). A few days after their arrival in the laboratory, fish were lightly anesthetized (clove oil, Omega Pharma, Plelo, France; 0.125 ml L^{-1}), weighed, measured (length, height, width) and tagged subcutaneously with a passive integrated transponder (PIT-tag; Ordicam, Rambouillet, France). Fish were acclimated to the laboratory conditions during 1 month. During that period they were fed *ad libitum* once to twice a week with a commercial feed (Le-Gouessant, Lambale, France) and were exposed to the natural day-night cycle.

All the fish were submitted to the protocol below and results were distinguished and organized according to individual PIT-tag number. Prior to environmental challenge tests, fish from both strains were brought together in the experimental arena and left undisturbed during 24h. This arena was identical to the rearing tanks with regard to location, size and water supply. Following recovery from the tests (4h) fish were returned to their original rearing tank.

Hypoxia challenge test (HCT)

HCT consisted of a rapid decrease in water oxygenation (from air saturation to 20 % air saturation in about one hour), followed by a much slower descent (approximately 2 % air saturation per hour) until the experiment ended (Fig.1). Ambient oxygenation was controlled by bubbling nitrogen in the input of a submersible pump placed in the tank. Nitrogen flow in the pump was manipulated using a controller and solenoid valve connected to a cylinder (Oxy-REG; Loligo Systems, Tjele, Denmark). As soon as a fish lost its ability to maintain balance *i.e.*, when the incipient lethal level (ILOS) was reached, it was quickly removed from the experimental arena, identified (pit tag reading) and placed in a fully aerated recovery tank. The corresponding time and oxygenation level was also recorded. Challenge ended as the last fish was removed from the experimental arena and it must be noted that less than 1 % mortality was observed following HCT.

Temperature challenge test (TCT)

Fish resumed feeding 24 h following HCT. Nevertheless, a minimum 7-day recovery period was allowed between HCT and TCT. TCT consisted of a period of rapid temperature increase (from acclimation to 27 °C in about 2.5 hours) followed by a slower increase (approximately 0.5 °C per hour) until the experiment ended (Fig.1). Water temperature was controlled using a 2500 W heater (JULABO, Seelbach, Germany). A submersible pump placed in the tank ensured water homogeneity and water oxygenation was maintained above 80 % air saturation via vigorous air bubbling. As fish lost equilibrium, they were quickly removed from the tank, identified (pit tag reading) and placed in a recovery tank at their

original acclimation temperature. The corresponding time and temperature (upper incipient lethal temperature, UILT) was also recorded. As for HCT, less than 1 % mortality was recorded in the days that followed TCT.

Swim tunnel respirometry

Swimming tests were conducted 1 month after the last HCT. They were carried out using a 30-L, swim-tunnel respirometer (Loligo Systems, Tjele, Denmark; swim chamber: 47 × 14 × 14 cm) supplied with the same water than the fish rearing tanks. The relationship between the rpm of the motor that propelled the water and the linear velocity of the water in the swimming chamber was established using a velocimeter (Höntzsch, Waiblingen, Germany). Fish oxygen consumption (MO_2) measurement sequence consisted of a period during which water supply to the tunnel was shut off (15 min) followed by a flushing period during which full oxygenation of the water was restored (5 min). During these sequences, oxygen saturation was always maintained above 80 % air saturation (% air sat). Water oxygenation was measured using an oxygen meter (Fibox 3; PreSens, Regensburg, Germany) connected to a computer. MO_2 was calculated as follows:

$$\dot{M}_{O2} = \frac{\Delta C_w O_2}{\Delta t} \times VOL_{resp} \times M^{-1}$$

where ΔC_wO_2 is the variation in water oxygen concentration (mgO_2 l^{-1}), Δt the duration of the measurement period (h), VOL_{resp} the volume of the respirometer minus the volume of fish (l) and M is fish body mass (kg).

Forty eight hours before swim tests were conducted, fish were placed in an acclimation chamber with the same dimensions as the swim chamber. Following this acclimatization period, animals were transferred into the swim-tunnel using a plastic bag filled with water to avoid emersion. Water velocity was set at 10 cm sec^{-1}, allowing fish to maintain position in the water current. The monitoring of water oxygenation was immediately initiated (sampling rate: 1 Hz). Fish were maintained in the swim tunnel for a total of 3 days. During the first two

days, fish acclimated to the respirometer and MO_2 was automatically monitored by connecting the water renewal pump (Eheim 1048, Germany) to a timer (Finder 80.91 0240 0000, Bever, Belgium). Standard metabolic rate (SMR) was calculated as the mean of the 10 lowest MO_2 measured between 00h and 07h during the second night of that period.

During the third day, fish were submitted to a standardized U_{crit} protocol in order to establish the relationship between metabolic rate and swimming speed. This protocol consisted in increasing water velocity by steps of 10 cm sec^{-1} every 20 min. At each step the corresponding MO_2 was determined twice using a cycle of 5-min measure followed by a 5-min flush (controlled manually). For the calculation of MO_2, only the last 4 minutes of each measuring period was used. The water velocity at which fish were no longer able to maintain position in the swimming chamber and rested on the posterior screen of the swimming chamber corresponded to the critical swimming speed (U_{crit}). The corresponding MO_2 was considered to indicate active metabolic rate (AMR). The aerobic metabolic scope (MS) was calculated as the difference between AMR and SMR. To account for the effect of the presence of fish on the velocity of the water in the swimming chamber, U_{crit} values were corrected using the following formula (Claireaux et al., 2006):

$$cU_{crit} = U_{crit} \times (1 + \varepsilon_s)$$

where cU_{crit} is the corrected maximum swimming speed, U_{crit} the observed maximum swimming speed and ε_s is a correction factor.

The correction factor ε_s was calculated as follows:

$$\varepsilon_s = (A \times B \times (L / ((W + H) / 2)) \times (CSA / T))^{1.5}$$

where L is the length of the fish, its width W and height H in cm and the CSA section of the fish in cm^2 and T the tunnel section in cm^2. A (here 0.8) and B (here 0.5) are coefficients taking into account the chamber geometry and fish shape respectively (Bell and Terhune, 1970).

As soon as U_{crit} was reached, water velocity was quickly reduced to 10 cm s^{-1} and a recovery period of 1 h was allowed before fish were removed from the swim-tunnel. Background bacterial oxygen consumption was then measured and systematically subtracted from fish MO_2. To avoid excessive bacterial colonization, the swim tunnel was cleaned with a bleach solution once a week. The oxygen probe was calibrated daily.

Morphometrics

As they were removed from the swim tunnel, fish were sacrificed by cerebral dislocation. The heart ventricle, the liver and the remaining viscera were excised, emptied, wiped on absorbent paper and weighed to the nearest hundredth of a gram. Gill arches were also dissected and gill lamellae carefully collected and weighed. The heart ventricle was placed in an ice-cold dish until processed for myofiberse oxygen consumption measurement (below).

Permeabilized ventricular myofibers oxygen consumption

The protocol below is adapted for trout from Toleikis et al. (1997). The ventricle was cut in three to four pieces which were weighed (range: 10 - 25 mg) and placed in 1.5 ml of ice-cold buffer (ATP: 1 mM, PCr: 2 mM, Dithiothreitol: 0.5 mM EDTA: 5.5 mM, $MgCl_2$: 2.5 mM, Imidazole: 10.0 mM, HEPES: 20.0 mM, KCl: 70.0 mM; pH 7.4) during less than 10 min to eliminate all traces of blood. Pieces of ventricle were then moved into 1.5 ml of chilled buffer with saponin (50 µg/ml) and collagenase (1.5 mg/ml). After 30 minutes, tissue fragments were then rinsed twice in cold buffer (10 min per rinse).

Oxygen consumption of the permeabilized ventricular myofibers was measured using a set of 6 polarographic oxygen electrodes and corresponding 10 °C-thermostatted, 2 ml glass respiration chambers (Strathkelvin Instruments Ltd, North Lanarkshire, Scotland) containing the respiration medium (TRIS: 20 mM, KCl: 150 mM, EDTA: 0.08 mM, NaH_2PO_4: 10 mM and $MgCl_2$: 7.5 mM; pH 7.2). As fragments of tissue were introduced in the chambers, a gentle stirring was initiated, together with the monitoring of the oxygen level in the respiration

medium. As soon as a steady state was reached (5 - 10 min), pyruvate, malate and ADP was injected in the respiration chamber at saturating concentrations (1 M, 0.5 M and 0.5 M respectively (Theron et al., 2000)). Maximal oxygen consumption of the permeabilized ventricular tissue (cMO_{2max}) was calculated using the slope of the decrease in the medium oxygenation level over time and was expressed as nmol O_2 min^{-1} g^{-1} wet tissue.

Data analysis and statistical analysis

Fish responses to challenge tests were expressed as time to loss of equilibrium, similar to time to death in survival studies, and were analyzed following procedures classically used for survival analysis. The relationship between the percentage of resisting individuals and time was estimated using the Kaplan-Meier procedure followed by a COX proportional hazards model to test for difference between strains (Cox F-test). The coefficient of variation (CV = standard deviation / mean) was used as an index of the extent of inter-individual variation. To generate mass-independent data of MO_2 (SMR and AMR), ILOS, IULT, U_{crit}, and organ-to-body mass ratios, residuals were calculated from least-squares linear regressions on body mass. If not stated otherwise, values are given as mean ±SEM, between strains comparisons were done using student t-test and statistical significance was set to $p < 0.05$. All statistical analyses were performed using Statistica-9 (Stat Soft).

RESULTS

Although being of the same age, experimental groups displayed a marked difference in body mass distribution ($p < 0.01$; Fig.2). In SGS body mass ranged between 73 and 182 g (mean: 119.95 ±6.41 g) whereas it ranged between 194 and 395 g (mean: 288.3 ±14.4 g) in FGS. Mean condition factor (M L^{-3}) was 1.29 ±0.17 in SGS and 1.62 ±0.21 in FGS ($p < 0.01$).

Table 1 summarizes among-strains comparison of the various parameters measured during this experiment.

246 Comparison of the time at which 50 % of the population has been removed from the
247 experimental arena (T_{50}) showed that FGS was more tolerant to hypoxia than SGS (T_{50} ≈
248 260 *versus* ≈ 200 min respectively; Cox F-test: $p < 0.01$; Fig.3a). Moreover, marked intra-
249 strain variability in individual responses to HCT was observed (Fig.3a). Time to loss of
250 equilibrium indeed ranged between 180 and 410 min for FGS and between 130 and 280 min
251 for SGS. This corresponded to incipient lethal oxygen saturation (ILOS) ranging from 13.4 to
252 16.7 % air sat in FGS and from 14.7 to 18.9 % air sat in SGS (Fig.3c).
253
254 Overall, SGS was found more tolerant to heat than FGS (T_{50} ≈ 400 *versus* ≈ 270 min
255 respectively; Cox F-test: $p < 0.01$; Fig 3b). As for HCT, response to TCT displayed
256 significant, within strain inter-individual variation (Fig.3b). However, this variability was more
257 marked for the FGS (160 min between the first and the last fish to lose equilibrium) than for
258 the SGS (30 min). This corresponded to incipient upper thermal limit (IULT) ranging from
259 24.7 to 27.6 °C in FGS and from 28.5 to 29.7 °C in SGS (Fig.3d). Although fish were allowed
260 a one-week recovery period between consecutive challenges, the possibility of an interaction
261 between performance during HCT and thermal tolerance (TCT) was examined and no
262 significant correlation between ILOS and IULT was found (data not shown). .
263
264 In both strains, active metabolic rate was highly correlated with body mass (Fig.4a; linear
265 regression, $p < 0.01$). On the other hand, SMR was found to increase with body mass in the
266 FGS (linear regression, $p < 0.01$) but not in the SGS (linear regression, $p < 0.33$). AMR
267 increasing much faster with body mass than SMR, the metabolic scope (MS) increased
268 significantly with mass. Over the whole size range, MS was increased nearly 7 times, ×2.2
269 within SGS and ×2.5 within the size range of FGS. Comparison of slopes showed no
270 differences between strains in the slopes of AMR *versus* body mass and SMR *versus* body
271 mass relationships ($p > 0.05$). Fitting a power model to the overall data set (Fig.4a; dotted-
272 hatched line) yielded a scaling exponent of 0.86 for SMR and 1.1 for AMR (Table 2).
273

No significant, within strain relationship between U_{crit} and body mass was found (Fig.4b; $p > 0.05$). However, significance emerged when the two experimental strains were combined ($p = 0.01$).

The mass of the ventricle, gills, liver and gut displayed significant positive relationships with body mass (Fig.5; linear regression, $p < 0.01$) and slope analysis showed that there was no statistically significant difference among strains ($p > 0.05$). Fitting a power model to the data showed that organ-mass-to-body-mass ratios increased as fish got bigger. Mass exponents were quite comparable, ranging from 1.18 for the gills to 1.23 for the gut (Table 2).

Analysis of residuals showed that neither organ-to-body-mass ratios, nor metabolic rates (SMR and AMR), nor swimming ability (U_{crit}) correlated with performance during environmental tolerance tests (HCT and TCT; data not shown). Conversely, maximal oxygen consumption of permeabilized myofibers (cMO_2) was found to be inversely related to heat tolerance ($p < 0.01$; Fig.6). However, no correlation between cMO2 and hypoxia tolerance (ILOS) was found (data not shown).

DISCUSSION

The main objective of the present study was to investigate determinants of environmental adaptation ability in a fish population faced with a combination of reduced oxygen availability and increased water temperature.

Body size and growth rate are obvious sources of inter individual variation. However, preliminary experiments had shown that, within an age cohort, variability in size was too limited to allow scrutinizing the interaction between growth rate and environmental tolerance with sufficient analytical power. To get around this difficulty, two lines of rainbow trout displaying marked differences in size at age were compared. Clearly, the main consequence of choosing this option was the confounding effects resulting from the difference in gene pool

between the two strains. Thus, in the present study, inter-strain comparison was specifically aimed at examining the relationship between body size and environmental adaptation performance, whereas intra-strain contrasting targeted size-independent sources of inter-individual variation.

Mean incipient lethal oxygen saturation (ILOS; 15-16 % air sat) and incipient upper lethal temperature (IULT; 28 - 30 °C) measured in the current study are consistent with available published data on salmonids. For instance, Galbreath et al. (2004) report IULT of 28 - 29 °C in 15 °C-acclimated rainbow trout (25 g). In much bigger Chinook salmon (*Oncorhynchus tsawytscha*; 2 - 5 kg), 25 °C corresponded to a limit above which cardiac arrhythmia indicated that fish were approaching their upper thermal tolerance (Clark et al., 2008). Field studies have also shown that 25 °C corresponded to a behavioral threshold above which rainbow trout were rarely observed (Matthews and Berg, 1996; Elliott, 2000). With regard to hypoxia, it has been established that 13 - 15 % air saturation corresponded to critical oxygen saturation of 10 °C-acclimated rainbow trout (Ott et al., 1980; Svendsen et al., 2012).

The degree of inter-individual variation in heat and hypoxia tolerance observed in the present study is worth specific attention. We indeed showed that the elapse time between the first and the last fish to lose equilibrium was approximately 5 h during the hypoxia challenge test and 4.5 h during the heat challenge test. This allowed us to precisely discriminate individual ILOS within the range 13 to 19 % air saturation (coefficient of variation; CV = 8.79 %) and IULT between 25 and 30 °C (CV = 4.95 %). Extensive individual variation in other complex performance traits has been reported in the literature (Millot et al., 2008; Nelson and Claireaux, 2005; Claireaux et al., 2007; Marras et al., 2011) and it has been proposed that CV ranging between 0 and 20 % are indicative of performance traits which have direct link with individual fitness (Webb, 1986; Reznick et al., 2004; Domenici, 2009). The small inter-individual variations in ILOS and IULT reported here are in line with the view that water oxygenation and temperature are potent determinants of Darwinian fitness in fish and that

tolerance to fluctuations in these environmental factors is maximized by natural selection (Somero, 2005; Mandic et al., 2009).

Experimental fish were of the same age (1 year) but originated from two different strains, a fast growing strain (FGS; mass = 290 g) and a slow growing strain (SGS; mass = 120 g). Comparison of heat and hypoxia tolerance in these strains showed that individuals from FGS displayed higher tolerance to reduced oxygen availability, whereas those issued from SGS were more tolerant to increased water temperature. For instance, Fig.3 shows that the time required to cull 50% of the population (T_{50}) during HCT was 260 min and 200 min for the FGS and SGS respectively, whereas during TCT, T_{50} values were 394 min and 264 min. Also worth noticing is the fact that the range of within strain variation in tolerance to hypoxia and hyperthermia was very similar to that observed at the species level *i.e.*, CV = 7 and 7 % (hypoxia) and 4 and 1 % (temperature) for FGS and SGS respectively.

Given that our experimental strains had been maintained under identical environmental conditions since hatching, and notwithstanding the genetic aspects discussed above, the most obvious traits liable to explain inter-strain difference in environmental adaptation ability are growth rate and body mass. Growth rate is known to trade off with a number of functions which include reproduction (Tsikliras et al., 2007), starvation tolerance (Prinet et al., 2010), risk taking (Killen, 2011), skeletal strength (Arendt and Wilson, 2000; Arendt et al., 2001) and swimming capacity (Farrell et al., 1997). However, published reports relating growth rate to environmental adaptation ability are scanty. Reduced ability to cope with hypoxia has been observed in genetically modified, fast-growing strain of *Oncorhynchus kisutch* (Sundt-Hansen et al., 2007). Similarly, *Oreochromis niloticus* selected for higher growth revealed lower cold tolerance than the non-selected fish (Rezk and Kamel, 2011). Conversely, Molony et al., (2004) showed that directed selection for faster growth was effective in selecting for increased heat tolerance in a line of rainbow trout. In the context of contemporary

environmental trends, the possible evolutionary outcome of the interaction between growth rate and environmental adaptation ability should deserve more attention.

Body mass is also a well recognized determinant of fish environmental adaptation ability. According to the literature, bigger individuals have a higher ability to survive in hypoxic conditions than smaller ones. Nilsson and Nilsson (2008) suggested that whereas the capacity of fish to extract environmental oxygen is independent of body mass, their ability to produce ATP anaerobically increases with size, small fish running out of glycogen or reaching lethal levels of metabolic end-products faster than big ones due to their higher mass-specific metabolic rate. In our study, within strain variation in fish size was too narrow to allow the detecting of any correlation between body mass and hypoxia tolerance. On the other hand, this relationship was found when hypoxia tolerances of FGS and SGS were combined (Fig.3c).

The difference in tolerance to heat that we observed between large and small individuals (Fig.3d) is also consistent with Clark et al. (2012). It has been proposed that thermal constraints on oxygen transport are responsible for setting the limit of heat tolerance (Pörtner and Knust, 2007). Given that cardiac capacity and aerobic metabolic scope are tightly related (Claireaux et al., 2005) and that high temperature limits maximum cardiac output (Farrell et al., 1996), a mismatch between oxygen requirements and the capacity of the cardiovascular system to transport oxygen is believed to be the primary functional level where thermal tolerance is set (Pörtner and Knust, 2007). Moreover, it has been shown, in salmonids, that the cardiovascular system was responsible for the inverse relationship between heat tolerance and body size. In these species, the ventricle indeed comprises an outer, compact layer which receives an arterial oxygen supply from the coronary circulation, and an inner, spongy myocardium which receives oxygen from the venous blood (Farrell, 1987). To our knowledge there is little published evidence that the perfusion of the compacta by coronaries changes with body size (but see Seierstad et al., 2008). On the other hand, it has been

385 demonstrated in Chinook salmon that increased body mass was accompanied by a lower
386 arterial and venous blood oxygen partial pressure (Clark et al., 2008). These authors
387 concluded that the poorer oxygenation of the myocardium through the spongiosa and
388 resulting decrease in cardiac performance was the cause of the lesser thermal tolerance of
389 bigger fish.
390
391 Field observations have confirmed that heat has stronger repercussions on large than on
392 small fish. The seasonal changes in the size distribution of the eelpout (*Zoarces viviparus*) in
393 the Wadden Sea have indeed been attributed to the higher summer mortality experienced by
394 the larger individuals (Pörtner and Knust, 2007). More recently, Eliason et al. (2011)
395 demonstrated that the migratory success of populations of sockeye salmon correlated with
396 the thermal sensitivity of their cardiac function. Residual analysis of maximum oxygen
397 consumption of permeabilized ventricular myofibers (cMO_2) brings an additional element in
398 support of the view that the cardiovascular system plays a key role in limiting heat tolerance.
399 As Fig.6 shows, individuals with lower cMO_2 also displayed higher thermal tolerance. Since
400 mitochondrial metabolism is the main source of cardiomyocyte oxygen demand, this result is
401 to be linked with the report of Pörtner et al. (2010) which shows that northern subspecies of
402 *Fundulus heteroclitus* had higher metabolic rate, higher mitochondrial oxygen consumption
403 and were more thermo-sensitive than more southern subspecies with reduced oxygen
404 demand. Differences in the number and functioning characteristics (capacities, coupling) of
405 the mitochondria could be involved in determining inter-individual variation in cMO_2.
406 However, these were not investigated in the present study.
407
408 Residues analysis revealed no correlation between cMO_2 and individual values of SMR,
409 AMR and U_{crit}. Additionally, no relationship was observed between SMR and any of the
410 organ-to-body-mass ratios. The lack of relationship between cMO_2 and complex performance
411 traits such as SMR and AMR may not be surprising considering that the myocardium
412 represents < 0.2 % of one fish body mass (Fig.5; Eliason et al., 2011) and that myocardium

oxygen consumption amounts to approximately 1 % of that of a whole fish (Ewart et al., 1988; Davie and Franklin, 1991). As discussed above, it is the diffusion of oxygen from the ventricular lumen into the thick-walled ventricle that determines heart working ability and not its aerobic metabolic performance *per se* (Farrell, 1991). This predominance of oxygen diffusion in determining cardiac performance is also to be linked with the absence of relationship between cMO_2 and U_{crit}.

The lack of relationship between organ-to-body mass ratios and SMR is counterintuitive as a higher cost of maintenance would be expected to derive from bigger organ (Wang et al., 2001; Suarez and Darveau, 2005). Confounding processes may have blurred the relationship that we anticipated. It has been observed that organ size is liable to fluctuate in relation with fish physiological (*e.g.*, sexual maturation) or nutritional (*e.g.*, feeding vs starving) status (Ghaffari et al., 2011; Kent et al., 1988; Franklin and Davie, 1992; Bailey et al., 1997; Sollid and Nilsson, 2006). These changes in organ size do not necessarily relate linearly with organ metabolic activity and this certainly contributes to obscure the relationships between organ mass and whole organism basal metabolic rate. This poor correspondence between organ mass and SMR may also explain why we found no significant correlation between organ-to-body-mass ratios and hypoxia or heat tolerance.

Examination of SMR, AMR and U_{crit} as a function of body mass showed that although growing at a different rate, the two strains followed the same scaling relationships (Fig.4). This similarity in scaling between strains was also observed when the various organ-to-body-mass ratios were considered (Fig.5). Scaling exponents for SMR (0.86; CI-95%: 0.69 - 1.04), AMR (1.10; CI-95%: 0.92 - 1.28) are consistent with published reports (Goolish, 1991; Clark and Johnston, 1999; Killen et al., 2007; Glazier, 2009) and they confirmed that these two metabolic states scale differently with body mass. It has been proposed that since resting metabolic rate is largely determined by energy-demand processes, O_2 transport and delivery contribute very little to the scaling of SMR. On the other hand, O_2 transport and delivery are

largely involved in determining maximal metabolism. As a result, their contribution to the scaling of AMR is significantly greater, hence augmented scaling exponent (Darveau et al., 2002).

Since AMR increased more rapidly with body mass than SMR, bigger fish were observed to have larger aerobic metabolic scope (MS) than smaller ones. MS represents an animal's capacity to support oxygen-consuming physiological functions (Fry, 1947). Our observation therefore suggests that small fish have less ability to multitask oxygen demanding processes than larger ones. The ecological implications of the scaling of metabolic scope with body size are nicely laid down in Killen et al. (2007). However, Pörtner and Knust (2007) and Pörtner et al. (2008) suggested that the size-specific thermal tolerance observed in adult fishes results from the decreasing aerobic scope with increasing body mass. Conversely, Clark et al. (2012) observed no change in aerobic metabolic scope with body mass in the Coho salmon (*Oncorhynchus kisutch*). This point deserves further investigation.

Critical swimming speed (U_{crit}) scaled with mass with an exponent of 0.29 ('CI-95%: 0.07 - 0.51). U_{crit} is a complex performance trait that involves an aerobic and an anaerobic component. Whereas aerobic energy production by red muscle tissue is predominant at slow speeds, it is gradually complemented by anaerobic metabolism by white muscle at higher speeds. These two components of U_{crit} do not scale in a similar fashion with body mass as aerobic swimming is more affected by body size than anaerobic swimming (Goolish, 1991). Moreover, anaerobic metabolism contributes a greater proportion of energy requirements to high-speed swimming in large fish than in small fish (Goolish, 1991; Clark et al., 2012). Whether these elements contribute to the observed scaling exponent remains elusive considering our relatively narrow size range. Future research will investigate the allometry of the relative contribution of aerobic and anaerobic metabolism to swimming performance.

468 The mass of the heart, liver, gills and gut scaled with body mass with exponents ≥ 1 (Table
469 2). These values are noticeably high but are in line with the report by Clark et al. (2012) in
470 Coho salmon (*Oncorhynchus kisutch*). Clark and Farrell (2011) report scaling exponent for
471 ventricle and liver of chinook salmon to be 0.95 and 0.84 respectively, whereas gill surface
472 area has been reported to scale with body mass with an exponent of 0.8 (Oikawa and
473 Itazawa, 1985; Palzenberger and Pohla, 1992). As already discussed above, our relatively
474 elevated scaling coefficients could be explained by the fact that one organ size is not simply
475 determined by the animal body size but is also influenced by exogenous (environmental) and
476 endogenous (physiological, nutritional) cycles. Among these, the sexual maturation cycle and
477 associated energy storage and mobilization is certainly the most relevant (Bon et al., 1997;
478 Franklin and Davie, 1992; Bailey et al., 1997). Obviously these changes are less likely to
479 affect the small, pre-pubescent animals than the large ones. Signs of sexual maturation were
480 observed in some individuals from the FGS group and this size-dependant interference may
481 have contributed to the exceedingly elevated inter-strain scaling exponents.
482
483 In conclusion, by comparing two genetically distinct strains of rainbow trout, we highlighted
484 differences in environmental tolerance between the fast growing and the slow growing
485 populations. Results also suggest a trade-off between thermal sensitivity and tolerance to
486 hypoxia. Size is a key element of that trade-off but functional, suborganismal components
487 are also involved. In the context of contemporary environmental trends, this sheds a new
488 light on the possible evolutionary response of fish populations faced with a warmer and less
489 oxygenated environment. However, discriminating genetic features from phenotypic plasticity
490 in the observed patterns will be a mandatory next step.
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496 ACKNOWLEDGEMENTS

497 Authors wish to thank Patrick Calves and Marc Belhomme for their valuable technical
498 assistance as well as Laurent Labbé (PEIMA-INRA) for providing the fish. Experiments were
499 conducted in accordance with applying French rules and regulations with regard to animal
500 care and experimentation.

501

502 REFERENCES

503 Arendt, J., Wilson, D.S., Stark, E., 2001. Scale strength as a cost of rapid growth in sunfish.
504 Oikos 93, 95-100.

505 Arendt, J.D., Wilson, D.S., 2000. Population differences in the onset of cranial ossification in
506 pumpkinseed (*Lepomis gibbosus*), a potential cost of rapid growth. Can. J. Fish. Aquat. Sci.
507 57, 351-356.

508 Bailey, J.R., West, J.L., Driedzic, W.R., 1997. Heart growth associated with sexual maturity
509 in male rainbow trout (*Oncorhynchus mykiss*) is hyperplastic. Comp. Biochem. Physiol. 118,
510 607-611.

511 Bell, W.H., Terhune, L.D.B., 1970. Water tunnel design for fisheries research. Fish. Res. Bd.
512 Can. Technical Report 195, 1-69.

513 Bon, E., Corraze, G., Kaushik, S., Le Menn, F., 1997. Effects of accelerated photoperiod
514 regimes on the reproductive cycle of the female rainbow trout: I-Seasonal variations of
515 plasma lipids correlated with vitellogenesis. Comp. Biochem. Physiol. 188, 183-190.

516 Claireaux, G., Couturier, C., Groison, A.-L., 2006. Effect of temperature on maximum
517 swimming speed and cost of transport in juvenile European sea bass (*Dicentrarchus*
518 *labrax*). J. Exp. Biol. 209, 3420-3428.

519 Claireaux, G., Dutil, J.-D., 1992. Physiological responses of the Atlantic cod (*Gadus morhua*)
520 to hypoxia at various environmental salinities. J. Exp. Biol. 163, 97-118.

521 Claireaux, G., Handelsman, C., Standen, E., Nelson, J.A., 2007. Thermal and temporal
522 stability of swimming performance in the European sea bass. Physiol. Biochem. Zool. 80,
523 186-196.

524 Claireaux, G., Lefrançois, C., 2007. Linking environmental variability and fish performance:
525 integration through the concept of scope for activity. Phil. Trans. R. Soc. 362, 2031-2041.

526 Claireaux. G., McKenzie, D.J., Genge, A.G., Chatelier, A., Aubin, J., Farrell, A.P., 2005.
527 Linking swimming performance, cardiac pumping ability and cardiac anatomy in rainbow
528 trout. J. Exp. Biol. 208, 1775-1784.

529 Clark, T.D., Donaldson, M.R., Pieperhoff, S., Drenner, M., Lotto, A., Cooke, S.J., Hinch, S.G.,
530 Patterson, D.A., Farrell, A.P., 2012. Physiological benefits of being small in a changing
531 world: responses of Coho salmon (*Oncorhynchus kisutch*) to an acute thermal challenge
532 and a simulated capture event. PLoS ONE 7, 1-8.
533 Clark, T.D., Farrell, A.P., 2011. Effects of body mass on physiological and anatomical
534 parameters of mature salmon: evidence against a universal heart rate scaling exponent.
535 J. Exp. Biol. 214, 887-893.
536 Clark, T.D., Sandblom, E., Cox, G.K., Hinch, S.G., Farrell, A.P., 2008. Circulatory limits to
537 oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus*
538 *tshawytscha*). Am. J. Physiol. 295, R1631-R1639.
539 Clarke, A., Johnston, N.M., 1999. Scaling of metabolic rate with body mass and temperature
540 in teleost fish. J. Animal Ecol. 68, 893-905.
541 Darveau, C.A., Suarez, R.K., Andrews, R.D., Hochachka, P.W., 2002. Allometric cascade as
542 a unifying principle of body mass effects on metabolism. Nature 417; 166-170.
543 Davie, P.S., Franklin, C.E., 1992. Myocardial oxygen consumption and mechanical efficiency
544 of a perfused dogfish heart preparation. J. Comp. Physiol. 162, 256-262.
545 Domenici, P., 2009. Escape responses in fish: kinematics, performance and behavior. In:
546 Domenici, P., Kapoor, B.mG. (Eds.), Fish Locomotion: An Eco-ethological Perspective.
547 Science Publishers, Enfield, pp. 123-170.
548 Dupont-Prinet, A., Chatain, B., Grima, L., Vandeputte, M., Claireaux, G., McKenzie, D., 2010.
549 Physiological mechanisms underlying a trade-off between growth rate and tolerance of feed
550 deprivation in the European sea bass (*Dicentrarchus labrax*). J. Exp. Biol. 213, 1143-1152.
551 Eliason, E.J., Clark, T.D., Hague, M.J., Hanson, L.M., Gallagher, Z.S., Jeffries, K.M., Gale,
552 M.K., Patterson, D.A., Hinch, S.G., Farrell, A.P., 2011. Differences in thermal tolerance
553 among sockeye salmon populations. Science 332: 109-112.
554 Elliott, J., 2000. Pools as refugia for brown trout during two summer droughts: trout
555 responses to thermal and oxygen stress. J. Fish Biol. 56, 938-948.

556 Ewart, H.S., Canty, A.A., Driedzic, W.R., 1988. Scaling of cardiac oxygen consumption and
557 enzyme activity levels in sea raven (*Hemitripterus americanus*). Physiol. Zool. 61, 50-56.
558 Farrell, A.P., 1987. Coronary flow in a perfused rainbow trout heart. J. Exp. Biol. 129, 107-
559 123.
560 Farrell, A.P., 1991. From Hagfish to Tuna: A Perspective on Cardiac Function in Fish.
561 Physiol. Zool. 64, 1137-1164.
562 Farrell, A.P., Gamperl, A., Hicks, J., Shiels, H., Jain, K., 1996. Maximum cardiac
563 performance of rainbow trout (*Oncorhynchus mykiss*) at temperatures approaching their
564 upper lethal limit. J. Exp. Biol. 199, 663-672.
565 Farrell, A.P., Bennett, W., Devlin, R.H., 1997. Growth-enhanced transgenic salmon can be
566 inferior swimmers. Can. J. Zool. 75, 335-337.
567 Ficke, A.D., Myrick, C.A., Hansen, L.J., 2007. Potential impacts of global climate change on
568 freshwater fisheries. Rev. Fish. Biol. Fisheries 17, 581-613.
569 Franklin, C.E., Davie, P.S., 1992. Sexual maturity can double heart mass and cardiac power
570 output in male rainbow trout. J. Exp. Biol. 171, 139-148.
571 Fry, F.E.J., 1947. Effect of the environment on animal activity. University Toronto Studies, 21
572 Biol. Ser. 55, 1-62.
573 Galbreath, P.F., Adams, N.D., Martin, T.H., 2004. Influence of heating rate on measurement
574 of time to thermal maximum in trout. Aquaculture 241, 587-599.
575 Ghaffari, H., Ardalan, A.A., Sahafi, H.H., Babaei, M.M., Abdollahi, R., 2011. Annual changes
576 in gonadosomatic index (GSI), hepatosomatic index (HIS) and condition factor (K) of
577 longuescale tongue sole *Cyboglossus arel* (Bloch & Schneider, 1801) in the coastal waters
578 of Bandar Abbas, Persian Gulf. Austr. J. Basic Appl. Sci. 5, 1640-1646.
579 Glazier, D.S., 2009. Activity affects intraspecific body-size scaling of metabolic rate in
580 ectothermic animals. J. Comp. Physiol. 179, 821-828.
581 Goolish, E.M., 1991. Aerobic and anaerobic scaling in fish. Biol. Rev. 66, 33-56.
582 Kent, J., Koban, M., Prosser, C.L., 1988. Cold-acclimation-induced protein hypertrophy in
583 channel catfish and green sunfish. J. Comp. Physiol. 158, 185-198.

584 Keyfitz, N., 1989. The growing human population. Scientific American 258, 71-78.
585 Killen, S.S., Costa, I., Brown, J.A., Gamperl, A.K., 2007. Little left in the tank: metabolic
586 scaling in marine teleosts and its implications for aerobic scope. Proc. R. Soc. B. 274, 431-
587 438.
588 Killen, S.S. 2011. Energetics of Foraging Decisions and Prey Handling. In: Farrell A.P., (ed.),
589 Encyclopedia of Fish Physiology: From Genome to Environment, volume 3, Academic
590 Press, San Diego, pp. 1588–1595.
591 Mandic, M., Todgham, A.E., Richards, J.G., 2009. Mechanisms and evolution of hypoxia
592 tolerance in fish. Proc. Roy. Soc. 276, 735-744.
593 Marras, S., Killen, S.S., Claireaux, G., Domenici, P., McKenzie, D.J., 2011. Behavioural and
594 kinematic components of the fast-start escape response in fish: individual variation and
595 temporal repeatability. J. Exp. Biol. 214, 3102-3110.
596 Matthews, K., Berg, N., 1997. Rainbow trout responses to water temperature and dissolved
597 oxygen stress in two southern California stream pools. J. Fish Biol. 50, 50-67.
598 Millot. S., Bégout, M.L., Person-Le Ruyet, J., Breuil, G., Di-Poï, C., Fievet, J., Pineau, P.,
599 Roué, M., Sévère, A., 2008. Feed demand behavior in sea bass juveniles: Effects on
600 individual specific growth rate variation and health (inter-individual and inter-group
601 variation). Aquaculture 274, 87-95.
602 Molony, B.W., Church, A.R., Maguire, G.B., 2004. A comparison of the heat tolerance and
603 growth of a selected and non-selected line of rainbow trout (*Oncorhynchus mykiss*), in
604 Western Australia. Aquaculture 241, 655-665.
605 Nelson, J.A., Claireaux, G., 2005. Sprint swimming performance of juvenile European sea
606 bass. Trans. Am. Fish. Soc. 134, 1274-1284.
607 Nilsson, G.E., Östlund-Nilsson, S., 2008. Does size matter for hypoxia tolerance in fish? Biol.
608 Rev. 83, 173-189.
609 Oikawa, S., Itazaawa, Y., 1985. Gill and body surface areas of the carp in relation to body
610 mass, with special reference to the metabolism-size relationship. J. Exp. Biol. 117, 1-14.

611 Ott, M.E., Heisler, N., Ultsch, G.R., 1980. A re-evaluation of the relationship between
612 temperature and the critical oxygen tension in freshwater fishes. Comp. Biochem. Physiol.
613 67, 337-340.
614 Palzenberger, M., Pohla, H., 1992. Gill surface area of water-breathing freshwater fish. Rev.
615 Fish Biol. Fish. 2, 187-216.
616 Pörtner, H.O., Bennett, A.F., Bozinovic, F., Clarke, A., Lardies, M.A., Lucassen, M., Pelster,
617 B., Schiemer, F., Stillman, J.H., 2006. Trade-Offs in Thermal Adaptation: The Need for a
618 Molecular to Ecological Integration. Physiol. Biochem. Zool. 79, 295-313.
619 Pörtner, H.O., Bock, C., Knust, R., Lannig, G., Lucassen, M., Mark, F.C., Sartoris, F.J., 2008.
620 Cod and climate in a latitudinal cline: physiological analyses of climate effects in marine
621 fishes. Climate Res. 37, 253-270.
622 Pörtner, H.O., Knust, R., 2007. Climate change affects marine fishes through the oxygen
623 limitation of thermal tolerance. Science 315, 95-97.
624 Pörtner, H.O., Schulte, P., Wood, C.M., Schiemer, F. 2010. Niche Dimensions in Fishes: An
625 Integrative View. Physiol. Biochem. Zool. 83, 808-826.
626 Rabalais, N.N., Turner, R.E., Díaz, R.J., Justić, D., 2009. Global change and eutrophication
627 of coastal waters. I.C.E.S. J. Mar. Sci. 66, 1528-1537.
628 Rezk, M.A., Kamel, E.A., 2011. Cold tolerance of selective breeding of *Oreochromis niloticus*
629 and *Oreochromis aureus*. Egyp. J. Aqua., 43-52.
630 Reznick, D.N., Bryant, M.J., Roff, D., Ghalambor, C.K., Ghalambor, D.E., 2004. Effect of
631 extrinsic mortality on the evolution of senescence in guppies. Nature 431, 1095-1099.
632 Schneider, S.H., 1989. The changing climate. Scientific American 261, 70-79.
633 Seierstad, S.L., Svindland, A., Larsen, S., Rosenlund, G., Torstensen, B.E., Evensen, Ø.,
634 2008. Development of intimal thickening of coronary arteries over the lifetime of Atlantic
635 salmon, *Salmo salar* L., fed different lipid sources. J. Fish Dis. 31, 401-13.
636 Sollid, J., Nilsson, G.E., 2006. Plasticity of respiratory structures-adaptive remodeling of fish
637 gills induced by ambient oxygen and temperature. Respir. Physiol. Neurobiol. 154, 241-251.

638 Somero, G.N., 2005. Linking biogeography to physiology: evolutionary and acclimatory
639 adjustments of thermal limits. Frontiers in Zoology 2, 1-9.
640 Suarez, R.K., Darveau, C.A., 2005. Multi-level regulation and metabolic scaling. J. Exp. Biol.
641 208, 1627-1634.
642 Sundt-Hansen, L., Sundström, L.F., Einum, S., Hindar, K., Fleming, I., Devlin, R., 2007.
643 Genetically enhanced growth causes increased mortality in hypoxic environments. Biology
644 Letters 3, 165-168.
645 Svendsen, J.C., Steffensen, J.F., Aarestrup, K., Frisk, M., Etzerodt, A., Jyde, M., 2012.
646 Excess posthypoxic oxygen consumption in rainbow trout (*Oncorhynchus mykiss*): recovery
647 in normoxia and hypoxia. Can. J. Zool. 90, 1-11.
648 Theron, M., Guerrero, F., Sebert, P., 2000. Improvement in the efficiency of oxidative
649 phosphorylation in the freshwater eel acclimated to 10.1 MPa hydrostatic pressure. J. Exp.
650 Biol. 203, 3019-3023.
651 Toleikis, A., Majiene, D., Trumbeckaite, S., Dagys, A., 1997. The effects of ischemia and
652 experimental conditions on the respiration rate of cardiac fibers. Mol. Cell. Biochem. 174,
653 87-90.
654 Tsikliras, A.C., Antonopoulou, E., Stergiou, K.I., 2007. A phenotypic trade-off between
655 previous growth and present fecundity in round sardinella *Sardinella aurita*. Popul. Ecol. 49,
656 221-227.
657 Wang, Z., O'Connor, T.P., Heshka, S., Heymsfield, S.B., 2001. The reconstruction of
658 Kleiber's law at the organ-tissue level. J. Nutr. 131, 2967-2970.
659 Webb, P., 1986. Locomotion and predator-prey relationships, In: Feder, M., Lauder, G.
660 (Eds.), Predator-prey relationships. Univ. Chicago Press, Chicago, pp. 24-42.
661

662 Table 1. Comparison of mean values for the main traits measured (*p*: statistical significance).
663 SGS: slow growing strain; FGS: fast growing strain. ILOS: incipient lethal oxygen
664 saturation; IULT: incipient upper lethal temperature; T50: time to cull 50% of the
665 experimental population during the hypoxia and temperature challenge tests; AMR active
666 metabolic rate; SMR: standard metabolic rate; U_{crit}: critical swimming speed; bl: body
667 length; cMO_2: ventricular myofibers oxygen consumption. NS: not statistically significant.
668

	SGS	FGS	*p*
Body mass (g)	119.95 ±6.41	288.30 ±14.40	< 0.01
Body length (cm)	20.98 ±0.41	26.04 ±0.49	< 0.01
ILOS (% air saturation)	16.44 ±0.30	14.90 ± 0.24	< 0.01
IULT (°C)	28.96 ±0.07	26.52 ±0.23	< 0.01
$T50_{hypoxia}$ (min)	200.00 ±4.01	260.55 ±10.07	< 0.01
$T50_{Temperature}$ (min)	400.01 ±5.07	270.44 ±9.12	< 0.01
AMR ($mgO_2\ h^{-1}\ kg^{-1}$)	495.64 ±34.78	531.65 ±28.93	NS
SMR ($mgO_2\ h^{-1}\ kg^{-1}$)	86.82 ±6.11	79.38 ±5.19	NS
U_{crit} cm sec^{-1}	78.57 ±5.55	98.19 ±4.85	<0.05
U_{crit} bl sec^{-1}	3.72 ±0.31	3.74 ±0.20	<0.05
Gill lamellae (g)	1.35 ±0.06	3.84 ±0.23	< 0.01
Liver (g)	1.1 ±0.03	3.05 ±0.18	< 0.01
Gut (g)	5.22 ±0.47	14.65 ±0.97	< 0.01
Heart ventricle (g)	0.14 ±0.01	0.40 ±0.03	< 0.01
cMO_2 (nmol $min^{-1}\ g^{-1}$)	862.96 ±46.63	1161.67 ±81.68	<0.01

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671

672 Table 2. Scaling coefficient (b) of organ mass to body mass and corresponding 95%
673 confidence interval (CI). Data from both strains are combined and correspond to the dotted
674 hatch line on Fig. 4 and 5 (n = 39).

675

	Gill	Liver	Gut	Ventricle	SMR	AMR	U_{crit}
b	1.18	1.20	1.23	1.19	0.86	1.1	0.29
CI	1.07-1.28	1.1-1.31	1.06-1.4	0.98-1.41	0.69-1.04	0.92-1.28	0.07-0.51

676
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678

679 FIGURE LEGENDS

680

681 Figure 1. Time course of water oxygenation (% air saturation) and temperature (°C) during
682 the hypoxia (panel a) and temperature (panel b) challenge tests.

683

684 Figure 2. Frequency distribution of body mass in the two experimental groups (bin size: 25
685 g). White bars: slow growing strain (n = 19). Black bars: fast growing strain (n = 20)

686

687 Figure 3. Kaplan-Meier probability plot of tolerance time during hypoxia challenge test (panel
688 a) and temperature challenge test (panel b). Solid line: fast growing strain; hatched line: slow
689 growing strain. Horizontal lines are meant to indicate time for culling half of the population
690 (T_{50}).
691 Panel c: relationship between body mass and incipient lethal oxygen saturation (ILOS = -
692 $0.005B_m$ + 16.718, r^2 = 0.14, p < 0.05). Panel d: relationship between body mass and
693 incipient upper lethal temperature (IULT = $-0.012B_m$ + 30.16, r^2 = 0.65, p < 0.05). Closed
694 symbols: fast growing strain, open symbols: slow growing strain.

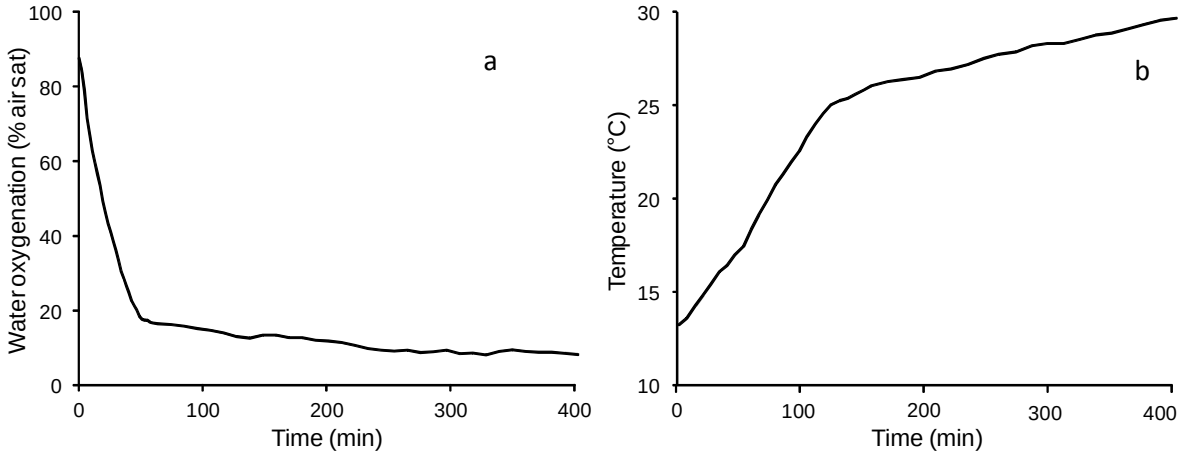
695

696 Figure 4. Panel a: relationships between rate of oxygen uptake (M_{O2}) and body mass (B_m).
697 Open symbols and hatched line: slow growing strain (SGS); closed symbols and solid line:
698 fast growing strain (FGS). Upper curves: active metabolic rate (AMR); lower curves: standard
699 metabolic rate (SMR). $AMR_{SGS} = 0.48B_m + 1.88$, $r^2 = 0.39$, $p = 0.004$; $SMR_{SGS} = 0.017B_m +$
700 7.99, $r^2 = 0.05$, $p = 0.33$; $AMR_{FGS} = 0.64B_m - 29.51$, $r^2 = 0.56$, $p < 0.000$; $SMR_{FGS} = 0.094B_m -$
701 3.95; $r^2 = 0.43$, $p = 0.001$.
702 Panel b: relationships between critical swimming speed (U_{crit}) and body mass (same symbols
703 as above). SGS: $U_{crit} = 0.13B_m + 62.85$; $r^2 = 0.02$; $p = 0.65$; FGS: $U_{crit} = 0.11B_m + 66.03$; $r^2 =$
704 0.11; $p = 0.22$.
705 Combined data (dotted hatch line): $SMR = 0.162B_m^{0.86}$, $r^2 = 0.73$; $AMR = 0.2985B_m^{1.10}$, $r^2 =$
706 0.80; $U_{crit} = 18.52B_m^{0.29}$, $r^2 = 0.23$.

Figure 5. Relationships between organ mass and body mass (B_m). Open symbols and hatched line: slow growing strain (SGS); closed symbols and solid line: fast growing strain (FGS). $Gill_{SGS} = 0.007B_m + 0.47$, $r^2 = 0.55$, $p < 0.000$; $Gill_{FGS} = 0.01B_m + 0.76$, $r^2 = 0.93$, $p < 0.000$. $Heart_{SGS} = 0.001B_m + 0.03$, $r^2 = 0.25$, $p = 0.03$; $Heart_{FGS} = 0.002B_m - 0.1452$, $r^2 = 0.62$, $p = 0.002$. $Gut_{SGS} = 0.05B_m - 0.95$, $r^2 = 0.49$, $p < 0.000$; $Gut_{FGS} = 0.05B_m + 1.16$, $r^2 = 0.44$, $p = 0.005$. $Liver_{SGS} = 0.009B_m – 0.85$, $r^2 = 0.75$, $p < 0.000$; $Liver_{FGS} = 0.012B_m -0.29$, $r^2 = 0.79$, $p < 0.000$. Combined data (dotted hatch line): $Gill = 0.005B_m^{1.18}$, $r^2 = 0.94$; $Ventricle = 0.0005B_m^{1.20}$, $r^2 = 0.79$; $Liver = 0.0035B_m^{1.20}$, $r^2 = 0.94$; $Gut = 0.014B_m^{1.23}$, $r^2 = 0.86$

Figure 6. Relationships between permeabilized cardiomyocytes maximum oxygen consumption (residuals; rcM_{O2}) and incipient upper lethal temperature (residuals; rIULT). Open symbols: slow growing strain (SGS); closed symbols: fast growing strain (FGS). $rIULT = -0.102rcM_{O2} - 3.93$, $r^2 = 0.25$, $p < 0.05$.

Figure 1


Water oxygenation (% air sat)
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80
60
40
20
0
0
100
200
300
400
Time (min)
a
Temperature (°C)
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100
200
300
400
Time (min)
b

730 Figure 2

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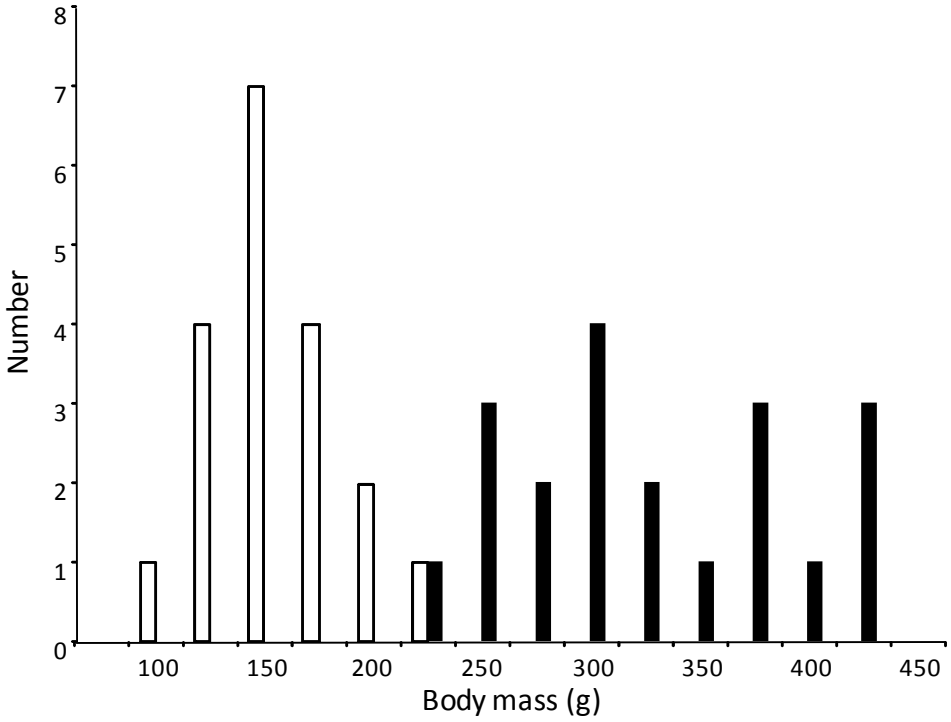

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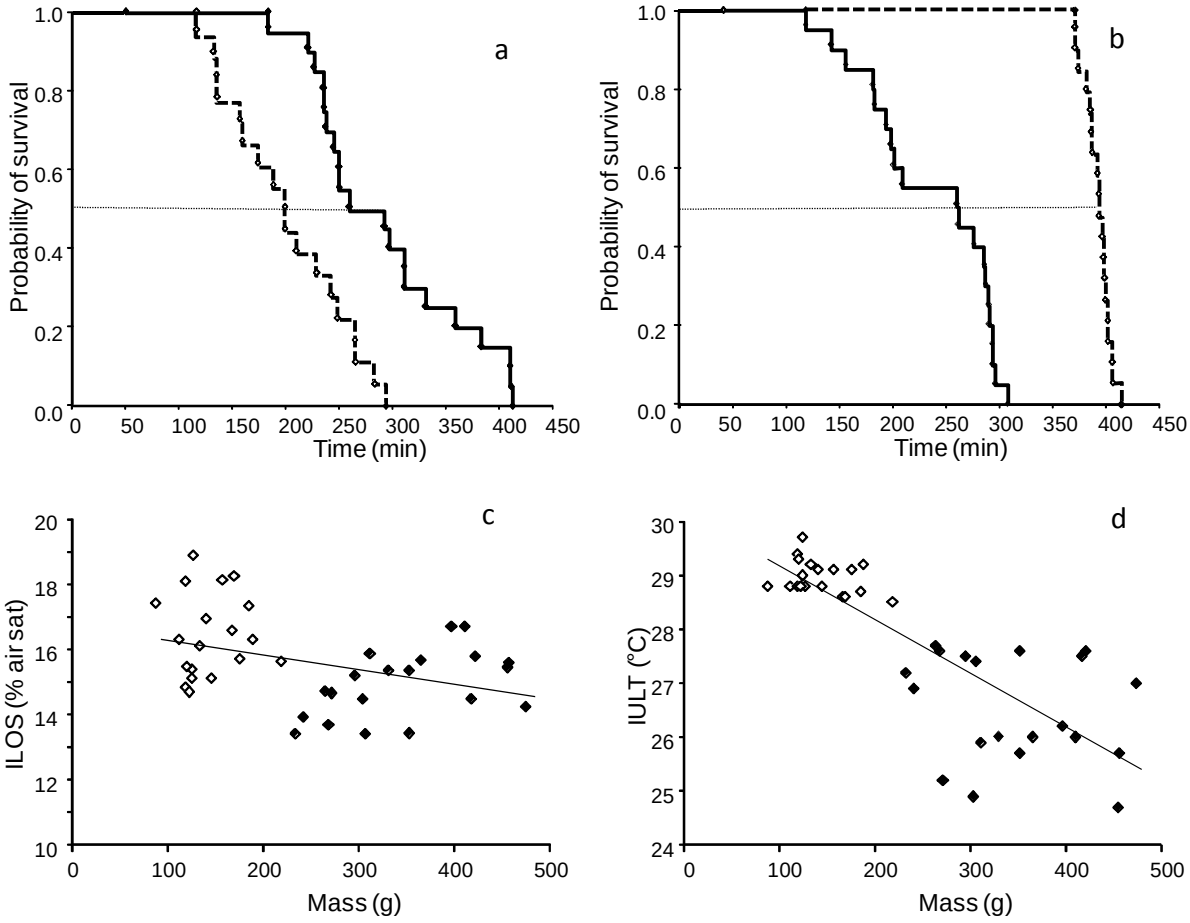
Figure 3


a
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Probability of survival
Time (min)
ILOS (% air sat)
IULT (°C)
Mass (g)

749 Figure 4

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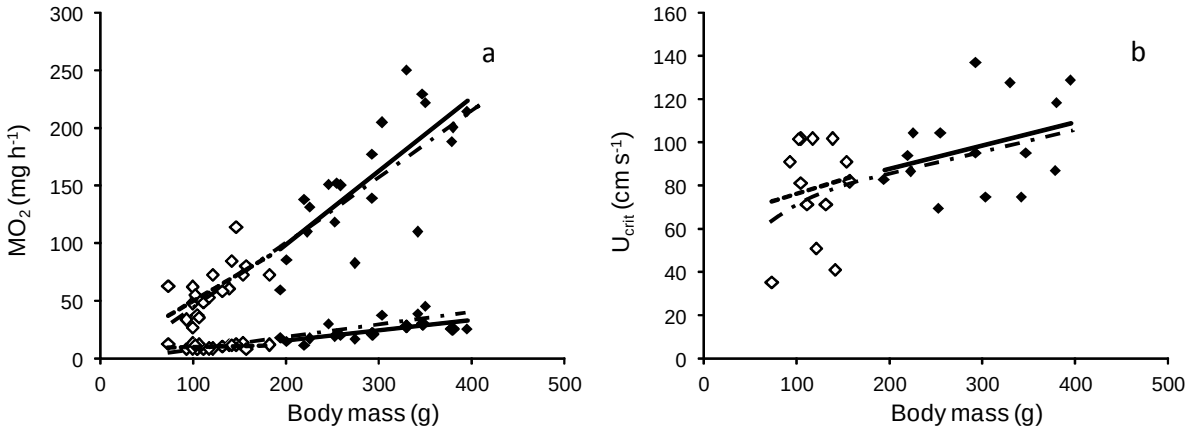

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759 Figure 5

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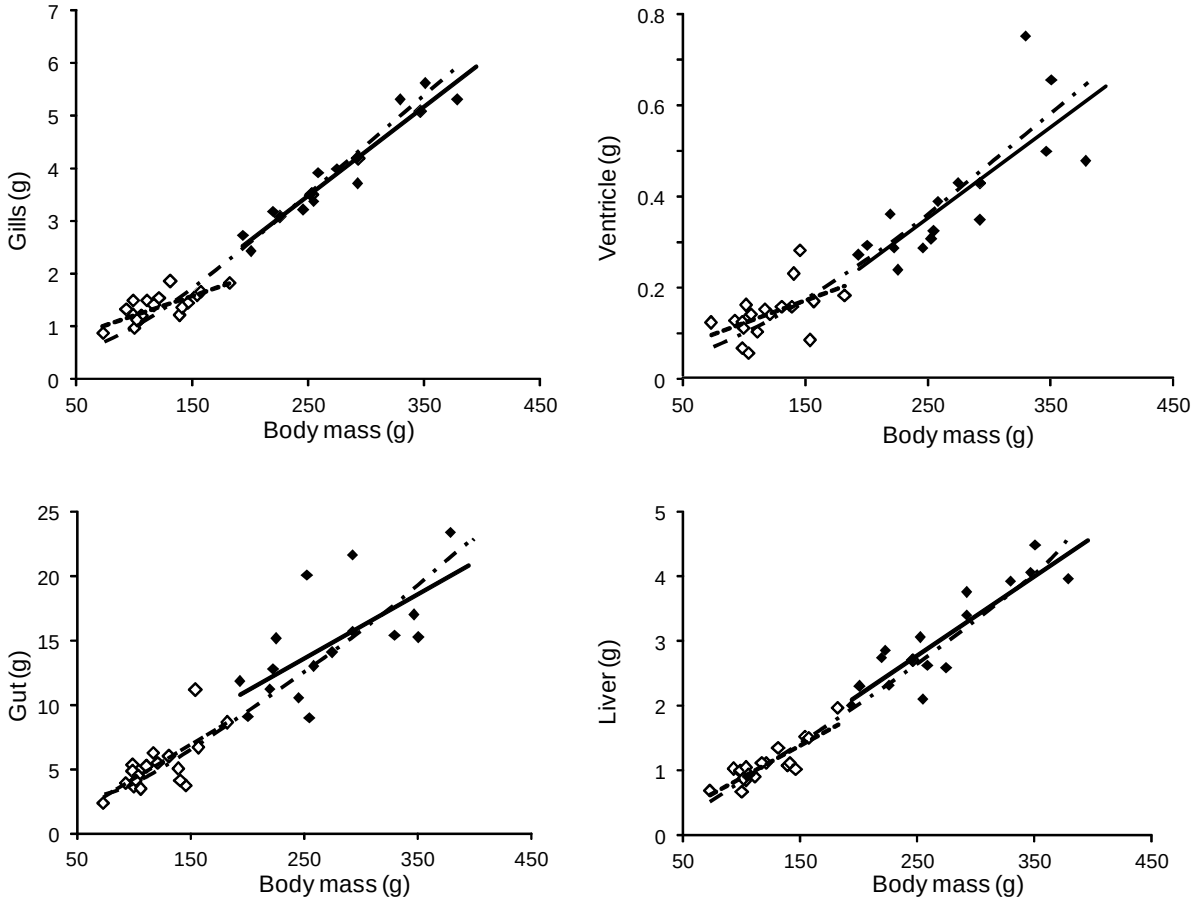

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762 Figure 6

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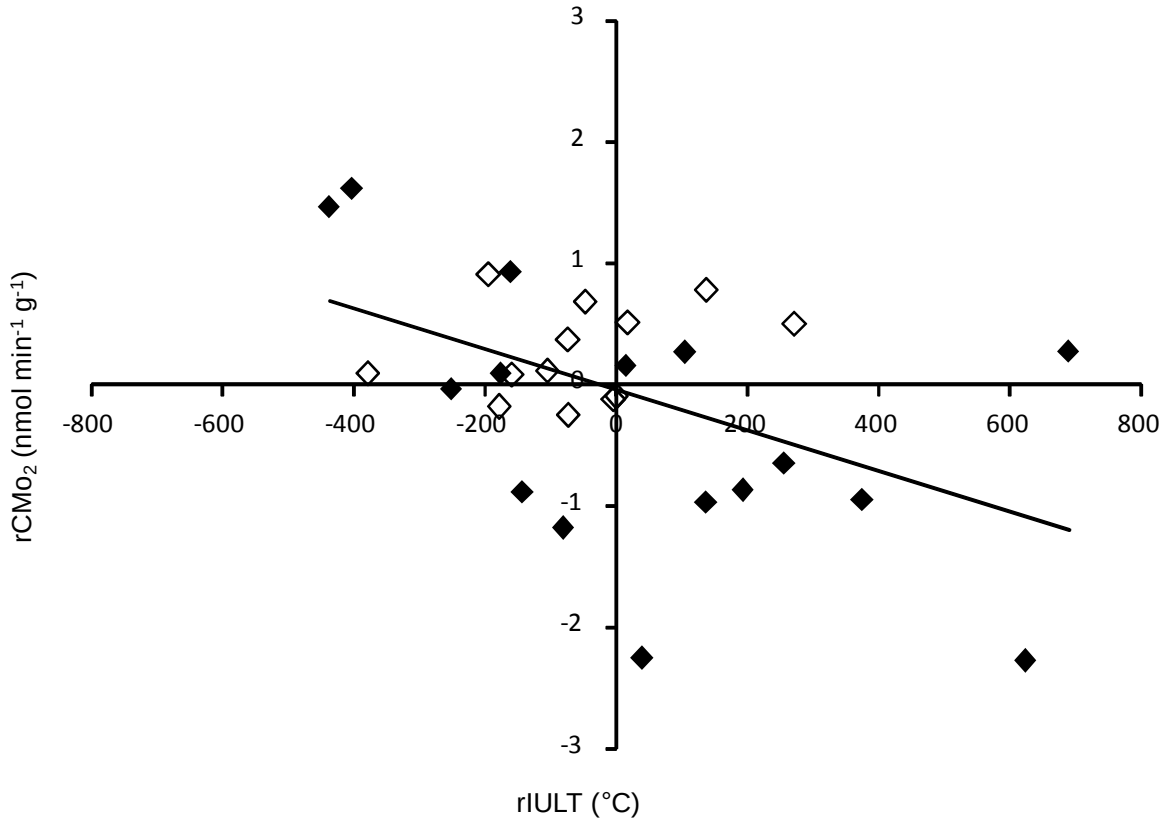

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