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 15\textsuperscript{th} 2009) and a slow growing strain (SGS;
hatching January 12\textsuperscript{th} 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\textsuperscript{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:

$$\dot{M}_{O_2} = \frac{\Delta C_wO_2}{\Delta t} \times VOL_{resp} \times M^{-1}$$

where $\Delta C_wO_2$ is the variation in water oxygen concentration (mgO$_2$ l$^{-1}$), $\Delta t$ 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 $U_{\text{crit}}$ 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 ($U_{\text{crit}}$). 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, $U_{\text{crit}}$ values were corrected using the following formula (Claireaux et al., 2006):

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

where $cU_{\text{crit}}$ is the corrected maximum swimming speed, $U_{\text{crit}}$ the observed maximum swimming speed and $\epsilon_s$ is a correction factor.

The correction factor $\epsilon_s$ was calculated as follows:

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

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 $U_{\text{crit}}$ 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 myofibers 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$_2$PO$_4$: 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$_{2\text{max}}$) 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, $U_{\text{crit}}$, 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.
Comparison of the time at which 50% of the population has been removed from the experimental arena ($T_{50}$) showed that FGS was more tolerant to hypoxia than SGS ($T_{50} \approx 260$ versus $\approx 200$ min respectively; Cox F-test: $p < 0.01$; Fig.3a). Moreover, marked intra-strain variability in individual responses to HCT was observed (Fig.3a). Time to loss of equilibrium indeed ranged between 180 and 410 min for FGS and between 130 and 280 min for SGS. This corresponded to incipient lethal oxygen saturation (ILOS) ranging from 13.4 to 16.7% air sat in FGS and from 14.7 to 18.9% air sat in SGS (Fig.3c).

Overall, SGS was found more tolerant to heat than FGS ($T_{50} \approx 400$ versus $\approx 270$ min respectively; Cox F-test: $p < 0.01$; Fig 3b). As for HCT, response to TCT displayed significant, within strain inter-individual variation (Fig.3b). However, this variability was more marked for the FGS (160 min between the first and the last fish to lose equilibrium) than for the SGS (30 min). This corresponded to incipient upper thermal limit (IULT) ranging from 24.7 to 27.6°C in FGS and from 28.5 to 29.7°C in SGS (Fig.3d). Although fish were allowed a one-week recovery period between consecutive challenges, the possibility of an interaction between performance during HCT and thermal tolerance (TCT) was examined and no significant correlation between ILOS and IULT was found (data not shown).

In both strains, active metabolic rate was highly correlated with body mass (Fig.4a; linear regression, $p < 0.01$). On the other hand, SMR was found to increase with body mass in the FGS (linear regression, $p < 0.01$) but not in the SGS (linear regression, $p < 0.33$). AMR increasing much faster with body mass than SMR, the metabolic scope (MS) increased significantly with mass. Over the whole size range, MS was increased nearly 7 times, $\times 2.2$ within SGS and $\times 2.5$ within the size range of FGS. Comparison of slopes showed no differences between strains in the slopes of AMR versus body mass and SMR versus body mass relationships ($p > 0.05$). Fitting a power model to the overall data set (Fig.4a; dotted-hatched line) yielded a scaling exponent of 0.86 for SMR and 1.1 for AMR (Table 2).
No significant, within strain relationship between $U_{\text{crit}}$ 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 ($U_{\text{crit}}$) 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
demonstrated in Chinook salmon that increased body mass was accompanied by a lower arterial and venous blood oxygen partial pressure (Clark et al., 2008). These authors concluded that the poorer oxygenation of the myocardium through the spongiosa and resulting decrease in cardiac performance was the cause of the lesser thermal tolerance of bigger fish.

Field observations have confirmed that heat has stronger repercussions on large than on small fish. The seasonal changes in the size distribution of the eelpout (Zoarces viviparus) in the Wadden Sea have indeed been attributed to the higher summer mortality experienced by the larger individuals (Pörtner and Knust, 2007). More recently, Eliason et al. (2011) demonstrated that the migratory success of populations of sockeye salmon correlated with the thermal sensitivity of their cardiac function. Residual analysis of maximum oxygen consumption of permeabilized ventricular myofibers (cMO$_2$) brings an additional element in support of the view that the cardiovascular system plays a key role in limiting heat tolerance. As Fig.6 shows, individuals with lower cMO$_2$ also displayed higher thermal tolerance. Since mitochondrial metabolism is the main source of cardiomyocyte oxygen demand, this result is to be linked with the report of Pörtner et al. (2010) which shows that northern subspecies of Fundulus heteroclitus had higher metabolic rate, higher mitochondrial oxygen consumption and were more thermo-sensitive than more southern subspecies with reduced oxygen demand. Differences in the number and functioning characteristics (capacities, coupling) of the mitochondria could be involved in determining inter-individual variation in cMO$_2$. However, these were not investigated in the present study.

Residues analysis revealed no correlation between cMO$_2$ and individual values of SMR, AMR and U$_{crit}$. Additionally, no relationship was observed between SMR and any of the organ-to-body-mass ratios. The lack of relationship between cMO$_2$ and complex performance traits such as SMR and AMR may not be surprising considering that the myocardium 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 ($U_{\text{crit}}$) scaled with mass with an exponent of 0.29 (CI-95%: 0.07 - 0.51). $U_{\text{crit}}$ 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 $U_{\text{crit}}$ 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.
The mass of the heart, liver, gills and gut scaled with body mass with exponents $\geq 1$ (Table 2). These values are noticeably high but are in line with the report by Clark et al. (2012) in Coho salmon (*Oncorhynchus kisutch*). Clark and Farrell (2011) report scaling exponent for ventricle and liver of chinook salmon to be 0.95 and 0.84 respectively, whereas gill surface area has been reported to scale with body mass with an exponent of 0.8 (Oikawa and Itazawa, 1985; Palzenberger and Pohla, 1992). As already discussed above, our relatively elevated scaling coefficients could be explained by the fact that one organ size is not simply determined by the animal body size but is also influenced by exogenous (environmental) and endogenous (physiological, nutritional) cycles. Among these, the sexual maturation cycle and associated energy storage and mobilization is certainly the most relevant (Bon et al., 1997; Franklin and Davie, 1992; Bailey et al., 1997). Obviously these changes are less likely to affect the small, pre-pubescent animals than the large ones. Signs of sexual maturation were observed in some individuals from the FGS group and this size-dependant interference may have contributed to the exceedingly elevated inter-strain scaling exponents.

In conclusion, by comparing two genetically distinct strains of rainbow trout, we highlighted differences in environmental tolerance between the fast growing and the slow growing populations. Results also suggest a trade-off between thermal sensitivity and tolerance to hypoxia. Size is a key element of that trade-off but functional, suborganismal components are also involved. In the context of contemporary environmental trends, this sheds a new light on the possible evolutionary response of fish populations faced with a warmer and less oxygenated environment. However, discriminating genetic features from phenotypic plasticity in the observed patterns will be a mandatory next step.
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Table 1. Comparison of mean values for the main traits measured ($p$: statistical significance).

SGS: slow growing strain; FGS: fast growing strain. ILOS: incipient lethal oxygen saturation; IULT: incipient upper lethal temperature; $T50$: time to cull 50% of the experimental population during the hypoxia and temperature challenge tests; AMR active metabolic rate; SMR: standard metabolic rate; $U_{crit}$: critical swimming speed; bl: body length; cMO$_2$: ventricular myofibers oxygen consumption. NS: not statistically significant.

<table>
<thead>
<tr>
<th>Trait</th>
<th>SGS</th>
<th>FGS</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (g)</td>
<td>119.95 ±6.41</td>
<td>288.30 ±14.40</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>20.98 ±0.41</td>
<td>26.04 ±0.49</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>ILOS (% air saturation)</td>
<td>16.44 ±0.30</td>
<td>14.90 ±0.24</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>IULT (°C)</td>
<td>28.96 ±0.07</td>
<td>26.52 ±0.23</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>$T50_{hypoxia}$ (min)</td>
<td>200.00 ±4.01</td>
<td>260.55 ±10.07</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>$T50_{Temperature}$ (min)</td>
<td>400.01 ±5.07</td>
<td>270.44 ±9.12</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>AMR (mgO$_2$ h$^{-1}$ kg$^{-1}$)</td>
<td>495.64 ±34.78</td>
<td>531.65 ±28.93</td>
<td>NS</td>
</tr>
<tr>
<td>SMR (mgO$_2$ h$^{-1}$ kg$^{-1}$)</td>
<td>86.82 ±6.11</td>
<td>79.38 ±5.19</td>
<td>NS</td>
</tr>
<tr>
<td>$U_{crit}$ cm sec$^{-1}$</td>
<td>78.57 ±5.55</td>
<td>98.19 ±4.85</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>$U_{crit}$ bl sec$^{-1}$</td>
<td>3.72 ±0.31</td>
<td>3.74 ±0.20</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Gill lamellae (g)</td>
<td>1.35 ±0.06</td>
<td>3.84 ±0.23</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>1.1 ±0.03</td>
<td>3.05 ±0.18</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Gut (g)</td>
<td>5.22 ±0.47</td>
<td>14.65 ±0.97</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Heart ventricle (g)</td>
<td>0.14 ±0.01</td>
<td>0.40 ±0.03</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>cMO$_2$ (nmol min$^{-1}$ g$^{-1}$)</td>
<td>862.96 ±46.63</td>
<td>1161.67 ±81.68</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Table 2. Scaling coefficient (b) of organ mass to body mass and corresponding 95% confidence interval (CI). Data from both strains are combined and correspond to the dotted hatch line on Fig. 4 and 5 (n = 39).

<table>
<thead>
<tr>
<th></th>
<th>Gill</th>
<th>Liver</th>
<th>Gut</th>
<th>Ventricle</th>
<th>SMR</th>
<th>AMR</th>
<th>U_{crit}</th>
</tr>
</thead>
<tbody>
<tr>
<td>b</td>
<td>1.18</td>
<td>1.20</td>
<td>1.23</td>
<td>1.19</td>
<td>0.86</td>
<td>1.1</td>
<td>0.29</td>
</tr>
<tr>
<td>CI</td>
<td>1.07-1.28</td>
<td>1.1-1.31</td>
<td>1.06-1.4</td>
<td>0.98-1.41</td>
<td>0.69-1.04</td>
<td>0.92-1.28</td>
<td>0.07-0.51</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

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

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

Figure 3. Kaplan-Meier probability plot of tolerance time during hypoxia challenge test (panel a) and temperature challenge test (panel b). Solid line: fast growing strain; hatched line: slow growing strain. Horizontal lines are meant to indicate time for culling half of the population (T_{50}).

Panel c: relationship between body mass and incipient lethal oxygen saturation (ILOS = -0.005B_m + 16.718, $r^2 = 0.14$, $p < 0.05$). Panel d: relationship between body mass and incipient upper lethal temperature (IULT = -0.012B_m + 30.16, $r^2 = 0.65$, $p < 0.05$). Closed symbols: fast growing strain, open symbols: slow growing strain.

Figure 4. Panel a: relationships between rate of oxygen uptake ($M_{O2}$) and body mass ($B_m$). Open symbols and hatched line: slow growing strain (SGS); closed symbols and solid line: fast growing strain (FGS). Upper curves: active metabolic rate (AMR); lower curves: standard metabolic rate (SMR). $AMR_{SGS} = 0.48B_m + 1.88$, $r^2 = 0.39$, $p = 0.004$; $SMR_{SGS} = 0.017B_m + 7.99$, $r^2 = 0.05$, $p = 0.33$; $AMR_{FGS} = 0.64B_m - 29.51$, $r^2 = 0.56$, $p < 0.000$; $SMR_{FGS} = 0.094B_m - 3.95$, $r^2 = 0.43$, $p = 0.001$.

Panel b: relationships between critical swimming speed ($U_{crit}$) and body mass (same symbols as above). SGS: $U_{crit} = 0.13B_m + 62.85$, $r^2 = 0.02$, $p = 0.65$; FGS: $U_{crit} = 0.11B_m + 66.03$, $r^2 = 0.11$, $p = 0.22$.

Combined data (dotted hatch line): $SMR = 0.162B_m^{0.86}$, $r^2 = 0.73$; $AMR = 0.2985B_m^{1.10}$, $r^2 = 0.80$; $U_{crit} = 18.52B_m^{0.29}$, $r^2 = 0.23$. 
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 = 0.005$B_m^{1.18}$, $r^2 = 0.94$; Ventricle = 0.0005$B_m^{1.20}$, $r^2 = 0.79$; Liver = 0.0035$B_m^{1.20}$, $r^2 = 0.94$; Gut = 0.014$B_m^{1.23}$, $r^2 = 0.86$

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

[Graph showing water oxygenation and temperature over time]
Figure 2

![Bar chart showing body mass distribution](#)

- **X-axis:** Body mass (g)
- **Y-axis:** Number

- Bars at 100, 150, 200, 250, 300, 350, 400, and 450 g show the number of occurrences of each body mass category.

- The highest number of occurrences is at 150 g, with a peak at 7.

- The lowest number of occurrences is at 350 g and 400 g, both with 1 occurrence.
Figure 3

Diagram a: Time (min) vs. Probability of survival

Diagram b: Time (min) vs. Probability of survival

Diagram c: Mass (g) vs. ILOS (% air sat)

Diagram d: Mass (g) vs. IULT (°C)
Figure 4

(a) MO$_2$ (mg h$^{-1}$) vs. Body mass (g)

(b) $U_{\text{crit}}$ (cm s$^{-1}$) vs. Body mass (g)
Figure 5

Body mass (g)

Liver (g)

Gut (g)

Gills (g)

Ventricle (g)
Figure 6

The graph shows a plot of \( r_{\text{CMO}_2} \) (nmol min\(^{-1}\) g\(^{-1}\)) against \( r_{\text{ULT}} \) (°C). The data points are scattered across the graph, and a linear trend line is fitted through the data, indicating a negative correlation between the two variables.