Inter-individual variation in mitochondrial phosphorylation efficiency predicts growth rates in ectotherms at high temperatures

Neal J. Dawson | Caroline Millet | Colin Selman | Neil B. Metcalfe

Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, UK

Correspondence
Neal J. Dawson, Institute of Biodiversity, Animal Health & Comparative Medicine, University of Glasgow, Graham Kerr Building, Glasgow G12 8QQ, UK.
Email: neal.dawson@gmail.com

Funding information
UKRI | Natural Environment Research Council (NERC), Grant/Award Number: NE/R001510/1;
Gouvernement du Canada | Natural Sciences and Engineering Research Council of Canada (NSERC), Grant/Award Number: PDF-488166-2016

Abstract
There is increasing evidence that aquatic ectotherms are especially vulnerable to global warming since their metabolic demands increase with ambient temperature while water-oxygen content decreases. The possible role of shrinking aerobic scope in limiting performance has been much discussed; however, less attention has been given to whether tissue-level changes in the efficiency of oxygen usage occur at elevated temperatures. Here, we show that this varies widely among individuals, with consequences for performance. We examined the inter-individual variation in growth rate and mitochondrial function from white muscle and liver of brown trout (Salmo trutta) acclimated to either high (19.5°C) or near-optimal temperature (12°C). Liver (but not muscle) mitochondria showed a positive relationship between growth rate and maximal oxidative phosphorylation at both temperatures, and a negative relationship between growth rate and ROS release. There was a positive correlation in both tissues between individual mitochondrial phosphorylation efficiency and growth rate, but only at 19.5°C. In this representative of aquatic ectotherms, an individual’s liver mitochondrial efficiency thus seems to dictate its capacity to grow at elevated temperatures. This suggests that individual heterogeneity in cellular function may cause variation in the thermal limits of aquatic ectotherms and could adversely affect wild populations in warming environments.

KEYWORDS
ATP/O, brown trout, climate change, global warming, liver, muscle

Abbreviations: ADP, adenosine diphosphate; COX, cytochrome oxidase/complex IV activity; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N′,N′-tetraacetic acid; LN, leak state respiration in the presence of pyruvate and malate, absence of ADP; LOmy, leak state respiration after the inhibition of the phosphorylation system by oligomycin; OXPHOS, oxidative phosphorylation; P_efficiency, net phosphorylation efficiency; PPM, OXPHOS respiration in the presence of pyruvate, malate and ADP; PPMG, OXPHOS respiration in the presence of pyruvate, malate, glutamate and ADP; PPMGS, OXPHOS respiration in the presence of pyruvate, malate, glutamate, succinate and ADP; RCR, respiratory control ratio; ROS, reactive oxygen species; Wf, final body mass; Wi, initial body mass.

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1 | INTRODUCTION

There is an increasing concern that climate change will cause potentially lethal increases in average water temperatures, highlighting the importance of understanding the effects of warming aquatic habitats on the physiology of aquatic ectotherms. Fish are of relevance here because their survival has previously been linked to their capacity to respond to thermal challenges. However, predicting the temperature at which populations of fish fail to survive has proven to be difficult to determine. While aerobic scope (the difference between maximum metabolic rate and standard metabolic rate) may be a good indicator of the upper thermal limits for some species, for many others it does not act as a reliable predictor. This means that some species begin to fail far below their expected upper thermal limits. How then can we reconcile the apparent failure of fish living at temperatures below their predicted upper thermal limit? One prevailing theory is that at high temperatures ectotherms will mature faster but reach maturity at a smaller body size. This is, in part, attributed to physiological responses that reduce metabolic requirements and thus enhance survival. However, extensive variation exists among individual animals of the same size, age and species in their capacity to grow near their upper thermal limit.

While the cause of this intraspecific variation in growth at high temperatures is not known, studies of ectotherms in other contexts have shown that individuals consuming similar amounts of food can experience nearly 3-fold variation in growth performance. Mitochondrial function may act as a potential determinant underlying ectotherm thermal tolerance, and animals can adjust the functioning of their mitochondria in response to prevailing conditions, although the response varies between individuals. One idea gaining momentum is that mitochondrial efficiency itself may act as a driver of the amount of food that an animal can consume or process. Recent work has shown that a link exists between mitochondrial efficiency and growth performance in brown trout at a temperature (12°C) close to the optimum for growth. In addition, the rate of proton leak in the mitochondria of brown trout correlates with whole-organism metabolic rate, suggesting that underlying mitochondrial efficiency may, in part, help dictate metabolic boundaries since a higher leak results in less efficient mitochondria, ultimately leading to poorer growth outcomes. However, higher mitochondrial leak might lead to less proton-coupling of the mitochondria, which has been associated with lower reactive oxygen species (ROS) production.

In addition to altering the level of mitochondrial coupling, animals can respond to changing metabolic demands by altering the volume of mitochondria within their cells. Fasting individuals have been shown to reduce mitochondrial volume, albeit with an increased whole-animal ROS burden; other studies have also found that reducing mitochondrial respiratory capacity seems to come at a cost in terms of oxidative stress. Therefore, changing either mitochondrial efficiency or mitochondrial volume can have complex consequences for organismal performance, which may explain the persistence of variation in these traits in aquatic animals.

Here we examine for the first time whether the extensive individual variation in growth rate observed in ectotherms at high temperatures is related to variation in mitochondrial capacity (maximum oxygen consumption rate) or efficiency (net phosphorylation efficiency), and what the consequences are for ROS release rates. To do this, we measured mitochondrial function in the white muscle of brown trout, which comprises the majority of body mass in fish, and in the liver, the organ responsible for metabolizing food. Our aim was to determine if increases in mitochondrial efficiency and/or capacity in one or both tissues could predict increases in food intake and growth of trout at high (19.5°C) and low temperatures (12°C), possibly at the cost of higher ROS release rates.

2 | MATERIALS AND METHODS

2.1 | Animal collection and husbandry

One year old immature brown trout (Salmo trutta; initial wet mass 19.38–63.27 g; fork length 119.1–172.0 mm; n = 51) were purchased from a commercial hatchery (Northern Trout Ltd, Ae Fishery, Dumfries, UK), and subsequently housed at the University of Glasgow where they were held under a 12 L: 12 D photoperiod for at least four weeks before any experiments were started. All animal husbandry and subsequent experiments were undertaken under UK Home Office project license P89482164 and following local ethical review. Under these conditions, fish were housed in groups in 1 m diameter plastic tanks connected to a recirculation system supplied with dechlorinated tap water at 14°C and fed daily ad libitum with fish pellets (Micro LR 15P BST (25/100); EWOS, Bathgate, UK). Fish were then split into two groups, one maintained at a water temperature of 12°C (n = 20) and the other at 19.5°C (n = 31; temperature was raised by –2°C/day) where they were held for 2 weeks.

Fish were then transferred in batches of 4 to individual compartments within two stream tank systems held at either 12°C (n = 20) or 19.5°C (n = 31) that allowed individual daily feeding while maintaining fish under the same water quality conditions. The fish were acclimated for one week in their individual compartments, during
which they were hand-fed daily to excess on trout pellets (Micro LR 15P BST (25/100); EWOS, Bathgate, UK). Fish were then fasted for 24 h and briefly anaesthetized (50 mg/L in 0.95% ethanol solution) for measurement of body mass (±0.01 g) and fork length (±0.01 g). Over the next 2 weeks, the fish were again hand-fed once daily on a ration of pellets that should result in satiation, using equations outlined in Elliott (1976) which determine the appropriate feeding rate. All fish were then fasted for 24 h and briefly anaesthetized (50 mg/L in 0.95% ethanol solution) for measurement of their body mass (W) and water temperature (T):

\[
\text{RCR} = \frac{1}{P_{\text{efficiency}}} = 1 - \frac{\text{Leak}}{\text{OXPHOS}}
\]

\[
12°C \text{ satiation ration} = 15.018 \times W^{0.759} e^{0.171 \times T}
\]

\[
19.5°C \text{ satiation ration} = 3.241 \times 10^7 \times W^{0.753} e^{-0.662 \times T}
\]

In all cases rations were confirmed to be in excess by observation. Fish were allowed to feed on their ration for 2 h in the morning, and excess pellets were then collected and counted. Food intake per day was determined by subtracting remaining pellets from the initial number added to each individual tank.

After the two-week growth trial the fish were humanely culled using an overdose of benzocaine (1 g L⁻¹ in 0.95% ethanol solution) and immediately weighed on an electronic balance (E2000D, Sartorius, Göttingen, Germany). White muscle and liver tissues were then excised within 2 min of death and transferred to 2 ml of ice-cold respiration buffer (in mmol L⁻¹: 20 Hepes, 0.5 EGTA, 3 MgCl₂, 60 potassium-lactobionate, 20 taurine, 10 KH₂PO₄ and 110 sucrose; with 1 mg ml⁻¹ fatty acid-free bovine serum albumin, pH 7.3) and gently homogenized with six passes of a dounce homogenizer at 100 rpm (Cole-Parmer PTFE Tissue Grinder, Cambridgeshire, UK).

2.2 Mitochondrial respiration

Mitochondrial function was measured (using a modified protocol of Salin et al.12) in 2 ml of respiration buffer using a high-resolution respirometer (Oxygraph-2k with O2k-Fluorescence module; Oroboros Instruments, Innsbruck, Austria) at the acclimation temperature (12 or 19.5°C) under continuous stirring. Liver (10 mg) or muscle (40 mg) tissue was allowed to sit for 5 minutes after being transferred to the chamber with the stirrer on. The amount of tissue added was optimized to a respiration level that was far below saturation levels for high resolution respirometry, but above any lower detection limits. Reactive oxygen species (ROS) were measured as described in Dawson et al.31 by the fluorescent detection of resorufin (excitation wavelength of 525 nm and AmR filter set, Oroboros Instruments). This was accomplished by adding exogenous superoxide dismutase (22.5 U ml⁻¹), Amplifi Red (15 μmol L⁻¹), and horseradish peroxidase (3 U ml⁻¹) to the respiration buffer. The rate of ROS emission was thus measured as the molar rate of Hydrogen peroxide (H₂O₂) appearance, using exogenous H₂O₂ (0.1 μmol L⁻¹) to calibrate the fluorescent resorufin signal. Respiration rate was determined from the rate of decline in O₂ concentration within the respirometry chamber; experimental runs were conducted at concentrations between 250 and 550 nmol/ml O₂ to ensure that oxygen limitation was not a factor.32 Full details of the protocol are given in the Supplementary Material 1, and only pertinent details given here. In the first step, respiration and ROS emission rates were measured after the addition of malate (2 mM) followed by pyruvate (5 mM; L_N). ADP (5 mM) was then added to stimulate respiration via complex I (termed P_PMF), then glutamate (10 mM) and finally succinate (25 mM) were added to determine the maximal capacity for supporting oxidative phosphorylation (maximum OXPHOS, P_PMG); then complexes I+II respectively. Cytochrome c (10 mM) was then added to assess the viability of our mitochondrial preparations (large increases in respiration following Cytochrome c additions are often used as an index of poor outer mitochondrial-membrane integrity).33,34 The addition of oligomycin was used to measure Leak state respiration (I_Om). Antimycin A revealed non-mitochondrial or background oxygen consumption, which was subtracted from all other measurements.

The respiratory control ratio (RCR) was calculated by calculating the ratio of respiration rate following addition of pyruvate, malate, glutamate, succinate and ADP (P_PMG) relative to the Leak respiration state (I_Om): addition of oligomycin). Net phosphorylation efficiency (P_efficiency) was calculated as described by Shama et al. (2016):35

\[
P_{\text{efficiency}} = 1 - \left(\frac{\text{Leak}}{\text{OXPHOS}}\right)
\]

\[
\text{Specific growth rate} = \left(\ln(W_f) - \ln(W_i)\right) \times (t^{-1}) \times 100
\]

where W_f and W_i refer to initial and final body mass and t = time elapsed in days.

Gross growth efficiency was calculated as described in Salin et al. (2019):31

\[
\text{Gross growth efficiency} = \frac{\text{final body mass} - \text{initial body mass}}{\text{food intake}}
\]
Gross growth efficiency = \( \frac{\text{mass gain} \times \text{day}^{-1}}{\text{mass of pellets eaten} \times \text{day}^{-1}} \) 
\hline

Food intake (expressed as % body mass per day) was calculated as described in Salin et al.\textsuperscript{4}.

Food intake = 100 \times \left( \frac{\text{mass of pellets eaten} \times \text{day}^{-1}}{W_i} \right) 
\hline

The differences in mean mitochondrial parameters between acclimation temperatures (12 vs. 19.5°C) were evaluated using two-tailed Student's \( t \)-test. We then used linear mixed models (R v.3.6.2; \texttt{http://www.R-project.org/}) to determine the relationships between individual variation in mitochondrial physiological parameters (P\textsubscript{efficiency}, maximal OXPHOS respiration (P\textsubscript{PMGS}) rates and ROS release in liver or muscle) and the specific growth rate of fish at 12 and 19.5°C. The full model included: specific growth rate as the dependent variable; P\textsubscript{efficiency}, maximal OXPHOS respiration, ROS release rates of liver and muscle, and initial body mass (at the start of the 2-week growth trial) as covariates; temperature (12 or 19.5°C) as a fixed factor; and two-way interactions between temperature and covariates. We also used a linear mixed model approach to test whether the measures of mitochondrial physiological function (P\textsubscript{efficiency}, maximal OXPHOS respiration rates and ROS release of the liver and/or muscle) explained individual variation in gross growth efficiency at both high and low temperatures. The full model included: gross growth efficiency as the dependent variable; P\textsubscript{efficiency}, maximal OXPHOS respiration, ROS release rates of liver and muscle, and initial body mass as covariates; temperature (12 or 19.5°C) as a fixed factor; and two-way interactions between temperature and covariates. Finally, we used the same approach to test whether the measures of mitochondrial physiological function (P\textsubscript{efficiency}, maximal OXPHOS respiration rates and ROS release of the liver and/or muscle) explained individual variation in food intake at both high and low temperatures. The full model included: food intake as the dependent variable; P\textsubscript{efficiency}, maximal OXPHOS respiration, ROS release rates of liver and muscle, and initial body mass as covariates; temperature (12 or 19.5°C) as a fixed factor; and two-way interactions between temperature and covariates. Processing batch was included as a random effect in all mixed models to control for the order in which fish were processed. All models were simplified by removing non-significant terms, starting with two-way interactions, and re-testing significance after each term was removed. Significance level was set to \( p < .05 \) in all statistical tests.

3 | RESULTS

3.1 | Effects of acclimation temperature on muscle and liver mitochondrial function

Mitochondrial respiratory capacities of white muscle and liver were significantly increased across all measured mitochondrial states in fish acclimated to 19.5°C compared to fish acclimated to 12°C (see Table 1 for Leak and maximum OXPHOS and Table S1 for other mitochondrial

| TABLE 1 | Mitochondrial properties (RCR, Net phosphorylation efficiency (P\textsubscript{efficiency}), Leak (L\textsubscript{Omy}) and maximum OXPHOS (P\textsubscript{PMGS}) respiration rates, ROS emission rates under P\textsubscript{PMGS} and the ratio of ROS emission to respiration rates under P\textsubscript{PMGS}) of muscle and liver from brown trout acclimated to either 19.5 or 12°C

| Parameter | Acclimation temperature (°C) | Muscle | Liver |
|-----------|-----------------------------|--------|-------|
|           | Mean                        | \( p \)-value | Mean | \( p \)-value |
| RCR       |                            |         |       |
| 19.5      | 7.95 ± 0.54                 | \( p = .67 \) | 10.84 ± 0.40* | \( p = .003 \) |
| 12        | 7.61 ± 0.59                 |         | 15.54 ± 1.35 |
| P\textsubscript{efficiency} |                            |         |       |
| 19.5      | 0.85 ± 0.014                | \( p = .50 \) | 0.90 ± 0.028  | \( p = .007 \) |
| 12        | 0.86 ± 0.010                |         | 0.93 ± 0.016 |
| L\textsubscript{Omy}       |                            |         |       |
| 19.5      | 0.85 ± 0.29*                | \( p < .0001 \) | 2.00 ± 0.10* | \( p < .0001 \) |
| 12        | 0.39 ± 0.05                 |         | 1.22 ± 0.08 |
| OXPHOS (P\textsubscript{PMGS}) |                            |         |       |
| 19.5      | 6.03 ± 0.27*                | \( p < .0001 \) | 21.19 ± 0.96* | \( p < .0001 \) |
| 12        | 2.62 ± 0.29                 |         | 14.91 ± 0.51 |
| ROS under OXPHOS (P\textsubscript{PMGS}) |                            |         |       |
| 19.5      | 0.0052 ± 0.0003*            | \( p < .0001 \) | 0.047 ± 0.003* | \( p < .0001 \) |
| 12        | 0.0028 ± 0.0002             |         | 0.030 ± 0.002 |
| Ratio of ROS/OXPHOS (P\textsubscript{PMGS}) |                            |         |       |
| 19.5      | 0.0009 ± 0.0003*            | \( p = .034 \) | 0.0022 ± 0.0008 | \( p = .35 \) |
| 12        | 0.0014 ± 0.0010             |         | 0.0020 ± 0.0007 |

Note: Values are given as the mean ± SEM (n = 20–31). * and bold font: significant pairwise differences between 19.5 and 12°C acclimation groups using a 2 tailed Student’s \( t \)-test (\( p < .05 \)).
This increase in respiration rate is an expected effect of temperature on mitochondrial function\textsuperscript{36}; however, our aim was to compare how among-individual variation in mitochondrial function affects growth at each temperature, not to compare average mitochondrial respiratory capacity at two different temperatures. The scale of the increase was similar in the two tissues, although liver tissue had higher respiration rates regardless of acclimation temperature when compared to white muscle (Tables 1 and S1). However, mitochondrial efficiency, represented by the respiratory control ratio (RCR), was reduced in the liver of warm-acclimated fish in comparison to cold-acclimated fish ($p = .003$; Table 1); a similar trend was seen with liver $P_{\text{efficiency}}$ ($p = .007$). There were no differences in white muscle mitochondria for either measure of efficiency (RCR, $p = .67$; $P_{\text{efficiency}}$, $p = .50$; Tables 1 and S1).

ROS release rates were increased in warm-acclimated compared to cold-acclimated fish under all respiratory states, with the increase being similar in both tissues except for only a marginal increase in ROS release during Leak respiration in liver mitochondria (Tables 1 and S1). The increases in ROS release from liver mitochondria in warm-acclimated fish were in proportion to the increases in liver mitochondrial respiration, since there was no significant overall effect of temperature on rates of ROS emission relative to $O_2$ consumption (ratio of ROS/OXPHOS ($P_{\text{PMGS}}$), $p = .35$; Table 1). However, white muscle mitochondria from warm-acclimated fish appear to show significantly lower ROS release rates relative to $O_2$ consumption when compared to cold-acclimated fish (ratio of ROS/OXPHOS ($P_{\text{PMGS}}$), $p = .034$; Table 1).

**FIGURE 1** Relationship between food intake and (A) net phosphorylation efficiency of liver mitochondria, (B) maximal OXPHOS respiration rate ($P_{\text{PMGS}}$) in liver mitochondria, (C) net phosphorylation efficiency of muscle mitochondria, (D) maximal OXPHOS respiration rate ($P_{\text{PMGS}}$) in muscle mitochondria of juvenile brown trout acclimated to high (19.5°C; $N = 31$) and low (12°C; $N = 20$) temperatures. Lines show significant effect for 12°C (solid line) and 19.5°C (dashed line). See Table 2 for statistical analyses.
3.2 Effects of mitochondrial function on food intake and growth parameters

Food intake was predicted by liver maximum OXPHOS rates ($P_{\text{PMGS}}$; higher rates being associated with higher food intake) and the interaction between liver P_efficiency and temperature: food intake was positively associated with liver P_efficiency but only at the higher temperature (Figure 1 and Table 1). Variation in the specific growth rate of the fish was in turn explained by liver maximum OXPHOS rates, liver ROS release rates, and the interaction between liver P_efficiency and temperature: the trends were similar to those for food intake, since OXPHOS was positively associated with growth, and liver P_efficiency only predicted growth rate at the higher temperature (Figure 2 and Table 2). Interestingly, variation in initial body mass among individuals was a poor predictor of growth rate ($p > .05$; Table 2; Figure S1). It is of note, that although it was not significant in our model, muscle P_efficiency correlated with growth rate when explored independently (Figure 2D; $R^2 = .141$; $p = .038$). Gross growth efficiency was predicted primarily by the interactions between temperature and both liver and muscle ROS release rates: thus liver and muscle ROS release rates only predicted gross growth efficiency at the lower temperature (Figure 3).

4 DISCUSSION

Our study shows that when food was freely available, the general trend regardless of acclimation temperature was for specific growth rate to increase with greater food intake; however, individuals exhibited markedly differing growth performance within each temperature group. In both warm and cold acclimated groups, variation in growth was strongly associated with liver mitochondrial function, where individuals that had higher liver mitochondrial respiratory capacity consumed more food and

**FIGURE 2** Relationship between specific growth rate and (A) net phosphorylation efficiency, (B) maximal OXPHOS respiration rate ($P_{\text{PMGS}}$), (C) ROS release rates ($P_{\text{PMGS}}$) of liver mitochondria, and (D) net phosphorylation efficiency of white muscle mitochondria in juvenile brown trout acclimated to high (19.5°C) and low (12°C) temperatures ($n = 31$ and 20 respectively). Lines show significant effects for 12°C (solid line) and 19.5°C (dashed line). See Table 2 for statistical analyses.
had a higher growth rate. Growth rate and efficiency also had an inverse relationship with mitochondrial ROS release. However, an individual’s mitochondrial efficiency seemed to determine growth rate only at the higher temperature-acclimation (19.5°C).

4.1 Food intake and growth rate are predicted by liver mitochondrial function

The food intake and growth rate of juvenile brown trout were largely driven by liver mitochondria respiratory capacity. This was seen across both warm and cold acclimation temperature groups. The liver in fish is the primary organ tasked with synthesizing proteins and molecules necessary for both the digestion of food and for the overall growth of an organism. It has been previously reported that higher liver mitochondrial capacity to produce ATP, required to drive digestion and food processing, is a key determinant of food intake by brown trout when given ad libitum access to food. In the same study, conducted at 12°C, it was found that food uptake was the key determinant of growth rate, rather than mitochondrial efficiency. Here, we also found little effect of mitochondrial efficiency on food uptake or growth rate at 12°C. However, at 19.5°C, we found that mitochondrial efficiency in the liver was a significant contributor to both growth rate and food uptake. In addition, liver mitochondrial efficiency (RCR) was on average lower in the warm-acclimated fish while oxygen consumption rates attributed to ATP production \((P_{PMGS})\) were higher. This would suggest that the efficiency with which brown trout liver is able to support growth via

### Table 2

Final models from linear mixed model analyses for specific growth rate, growth efficiency and food intake of brown trout as a function of acclimation temperature and mitochondrial properties in muscle and liver (net phosphorylation efficiency \((P_{efficiency})\), maximum OXPHOS respiration rate \((P_{PMGS})\) and ROS release rates during maximum OXPHOS)

| Dependent variable | Source of variation | Parameter estimate ± SE | Statistical results |
|--------------------|---------------------|-------------------------|---------------------|
| Food intake \(^d\) | Intercept \(^b\) | -5.499 ± 12.011 | \(F_{7,44} = 4.056, p = .046\) |
|                   | Temperature \(^b\)  | -39.34 ± 19.175        | \(F_{7,44} = 0.227, p = .640\) |
|                   | Liver \(P_{efficiency}\) | 6.817 ± 14.465 | \(F_{7,44} = 22.156, p < .0001\) |
|                   | Liver OXPHOS \((P_{PMGS})\) | 0.913 ± 0.192 | \(F_{7,44} = 4.814, p = .031\) |
|                   | Temperature \(\times\) Liver \(P_{efficiency}\) | 52.0398 ± 23.3607 | \(F_{7,44} = 4.913, p = .006\) |

| Specific growth rate \(^a\) | Intercept \(^b\) | 1.243 ± 3.722 | \(F_{6,41} = 5.308, p = .026\) |
| (Temperature \(\times\) Liver \(P_{efficiency}\)) | -14.702 ± 6.381 | \(F_{6,41} = 0.035, p = .851\) |
| (Liver OXPHOS \((P_{PMGS})\)) | -0.758 ± 4.023 | \(F_{6,41} = 18.344, p = .0001\) |
| (Liver ROS release \((P_{PMGS})\)) | 0.0747 ± 0.0174 | \(F_{6,41} = 4.343, p = .043\) |
| (Temperature \(\times\) Liver ROS release \((P_{PMGS})\)) | -11.857 ± 5.691 | \(F_{6,41} = 5.664, p = .022\) |
| (Temperature \(\times\) Muscle ROS release \((P_{PMGS})\)) | 16.756 ± 7.041 | \(F_{6,41} = 5.401, p = .019\) |

| Growth efficiency \(^c\) | Intercept \(^b\) | 0.139 ± 0.015 | \(F_{8,27.1} = 15.366, p < .001\) |
| Temperature \(^b\) | -0.073 ± 0.019 | \(F_{8,27.1} = 1.885, p = .178\) |
| Muscle ROS release \((P_{PMGS})\) | -5.035 ± 3.668 | \(F_{8,27.6} = 10.388, p < .003\) |
| Liver ROS release \((P_{PMGS})\) | -1.133 ± 0.352 | \(F_{8,27.6} = 8.329, p = .006\) |
| (Temperature \(\times\) Muscle ROS release \((P_{PMGS})\)) | 11.976 ± 4.149 | \(F_{8,27.6} = 5.803, p = .022\) |
| (Temperature \(\times\) Liver ROS release \((P_{PMGS})\)) | 0.967 ± 0.401 | \(F_{8,27.1} = 5.308, p = .026\) |

Note: Processing batch was included in all models as a random effect to control for the order in which fish were processed. Non-significant terms were excluded from the final models except when involved in significant interactions. Bold denotes significant terms.

\(^a\) Full model: specific growth rate = temperature + initial body mass + liver net phosphorylation efficiency + muscle net phosphorylation efficiency + liver \(P_{PMGS}\) + muscle \(P_{PMGS}\) + Liver ROS + Muscle ROS + (temperature \(\times\) liver \(P_{PMGS}\)) + (temperature \(\times\) muscle \(P_{PMGS}\)) + (temperature \(\times\) initial body mass) + (temperature \(\times\) liver ROS) + (temperature \(\times\) muscle ROS) (Table S1).

\(^b\) Temperature: two-level fixed factor (low and high temperature).

\(^c\) Full model: growth efficiency = temperature + initial body mass + liver net phosphorylation efficiency + muscle net phosphorylation efficiency + liver \(P_{PMGS}\) + muscle \(P_{PMGS}\) + Liver ROS + Muscle ROS + (temperature \(\times\) liver \(P_{PMGS}\)) + (temperature \(\times\) muscle \(P_{PMGS}\)) + (temperature \(\times\) initial body mass) + (temperature \(\times\) liver ROS) + (temperature \(\times\) muscle ROS) + (temperature \(\times\) liver ROS) + (temperature \(\times\) muscle ROS) (Table S2).

\(^d\) Full model: Food intake = temperature + initial body mass + liver net phosphorylation efficiency + muscle net phosphorylation efficiency + liver \(P_{PMGS}\) + muscle \(P_{PMGS}\) + Liver ROS + Muscle ROS + (temperature \(\times\) liver \(P_{PMGS}\)) + (temperature \(\times\) muscle \(P_{PMGS}\)) + (temperature \(\times\) initial body mass) + (temperature \(\times\) liver ROS) + (temperature \(\times\) muscle ROS) (Table S2).
ATP production is decreasing at higher temperatures. The reduced oxygen availability in water at higher temperatures, coupled with increased minimal metabolic demands, may place a greater emphasis on mitochondrial efficiency in the face of warming temperatures.

Previous work on brown trout reported that liver mitochondrial efficiency and muscle mitochondrial density (cytochrome c oxidase activity) were indicators of growth performance, however, that work was done under temperature conditions (12°C) close to the optimal for growth. Although it was found to be non-significant when incorporated into our models of growth performance, it is of note that muscle net phosphorylation efficiency was positively correlated with growth rates in warm, but not cold, acclimated fish, similar to that in the liver. This may suggest that the importance of mitochondrial efficiency for growth may depend on energetic demands (being significant when food is limiting or under high temperatures).

Interestingly, liver mitochondrial ROS release rates under P<sub>PMGS</sub> showed a negative relationship with growth rate. Liver ROS release rates were higher at 19.5°C than at 12°C, suggesting that the higher liver mitochondrial capacity at the warmer acclimation temperature may come at the cost of greater ROS release. However, both the rate of ROS release and the ratio of ROS production to OXPHOS respiration varied considerably (approximately 4-fold and 6-fold, respectively) among fish, which opens the possibility that perhaps some individuals have both more efficient liver mitochondria and a greater capacity to buffer mitochondrial ROS production.

### 4.2 Growth efficiency is predicted by lower mitochondrial ROS release rates at cold temperatures

Curiously, greater growth efficiency was predicted by lower mitochondrial ROS release rates in both tissues of the cold acclimated group, but not in the warm acclimated group. Warm acclimated fish did show higher ROS release rates overall, and the majority of warm-acclimated individuals had ROS release rates that were greater than the highest values seen in the cold-acclimated group. This could suggest that efficiency of mitochondrial ROS detoxification impacts growth efficiency, but only below a specific threshold. Fish liver is tasked with detoxifying a wide range of potentially harmful biomolecules, including ROS, and liver antioxidant enzymes are often used as a biomarker of environmental contaminants. Therefore, it is reasonable that as ROS release rates increase in liver mitochondria, more of the ATP produced will go towards detoxification. ROS release rates in the muscle were higher at 19.5°C than 12°C in terms of absolute values, but release rates relative to oxygen consumption were actually lower. This is very interesting since it suggests a temperature-specific relationship with mitochondrial ROS release rates. This is in line with previous work on a related species (Salmo salar) showing that cardiac mitochondria reduce ROS release rates at high temperatures (20–28°C).

Muscle has also been found to be fairly plastic in its response to increased ROS production, showing a remarkable ability to adjust its antioxidant capacity in response to increases in ROS exposure.

### 4.3 Conclusions

In conclusion, our study has demonstrated that at high temperature (19.5°C) there is a positive relationship...
between liver mitochondrial function and growth performance of brown trout. High growth rates at warm temperatures seem to come at the cost of increased ROS release rates relative to those seen at lower temperatures, but the fastest growth for brown trout is exhibited by those individuals with both higher mitochondrial efficiency and lower ROS release rates. Future work should focus on how such individuals can maintain efficient mitochondrial function while minimizing ROS release. This study, combined with previous work, seems to suggest that when food availability is high, liver mitochondrial capacity may dictate growth outcomes, while growth in the face of stressful conditions (e.g., high temperature, lower food availability) may favor those individuals with more efficient mitochondria. What remains to be seen is what costs are associated with a faster growth rate. Does an accelerated growth lead to a shortened lifespan, as some previous works suggests (see Metcalfe and Monaghan), will individuals with more efficient mitochondria have higher ROS loads leading to an accumulation of ROS-based oxidative damage, or will warming aquatic habitats result in the selection for only the most energetically efficient individuals? Brown trout, like other freshwater fish, are generally unable to move their habitat range; therefore, answering these questions is vital if we are to prioritize populations for protection measures, or to select sites for conservation translocation.

ACKNOWLEDGEMENTS

We would like to thank the animal care team at the Institute of Biodiversity, Animal Health and Comparative Medicine for help with fish husbandry: Toby Miller, Alastair Kirk, Ross Phillips and Graham Law. This research was supported by a grant from the Natural Environment Research Council (NERC; no. NE/R001510/1 to N.B.M, C.S., Richard C. Hartley and Pat Monaghan), and by Advanced Grant 834653 from the European Research Council to N.B.M.. N.J.D. was also supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Postdoctoral Fellowship.

DISCLOSURES

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Neal J. Dawson, Caroline Millet and Neil B. Metcalfe conceived of the study and designed the protocol. Neal J. Dawson performed the experimental and laboratory work. Neal J. Dawson analyzed the data, produced the graphic material, and wrote the first draft. This was reviewed and edited by all authors. Neil B. Metcalfe and Caroline Millet procured the funding. All authors approved the final version of the manuscript and agreed to be accountable for all contents.

DATA AVAILABILITY STATEMENT

The data used in this study can be found in the Supporting Information (Supplementary Material 2).

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Additional supporting information may be found in the online version of the article at the publisher’s website.

**How to cite this article:** Dawson NJ, Millet C, Selman C, Metcalfe NB. Inter-individual variation in mitochondrial phosphorylation efficiency predicts growth rates in ectotherms at high temperatures. *FASEB J*. 2022;36:e22333. doi:[10.1096/fj.202101806RR](https://doi.org/10.1096/fj.202101806RR)