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RESEARCH ARTICLE

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

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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′,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.

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.[1–3] Fish are of relevance here because their survival has previously been linked to their capacity to respond to thermal challenges.[4–6] 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.[7,8] 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.[9,10] This is, in part, attributed to physiological responses that reduce metabolic requirements and thus enhance survival.[11] However, extensive variation exists among individual animals of the same size, age and species in their capacity to grow near their upper thermal limit.[12]

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.[13,14] Mitochondrial function may act as a potential determinant underlying ectotherm thermal tolerance.,[15–18] and animals can adjust the functioning of their mitochondria in response to prevailing conditions, although the response varies between individuals.[19] 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.[12,20–22] 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.[21] 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[23] since a higher leak results in less efficient mitochondria, ultimately leading to poorer growth outcomes.[21] However, higher mitochondrial leak might lead to less proton-coupling of the mitochondria, which has been associated with lower reactive oxygen species (ROS) production.[24–26]

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[27]; other studies have also found that reducing mitochondrial respiratory capacity seems to come at a cost in terms of oxidative stress.[28,29] 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.[19]

Here we examine for the first time whether the extensive individual variation in growth rate observed in ectotherms at high temperatures[21] 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.[27] 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)[30] which allow calculation of individual-specific rations in calories as a function of the fish's body mass (W) in grams and water temperature (T):

$$12°C\ \text{satiation ration} = 15.018 \times W^{0.759} e^{0.171 \times T} \quad (1)$$

$$19.5°C\ \text{satiation ration} = 3.241 \times 10^{7} \times W^{0.753} e^{-0.662 \times T} \quad (2)$$

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 2ml of ice-cold respiration buffer (in mmol L^{-1}: 20 Hepes, 0.5 EGTA, 3 $MgCl_2$, 60 potassium-lactobionate, 20 taurine, 10 KH_2PO_4 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}), Ampliflu 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 (H_2O_2) appearance, using exogenous H_2O_2 (0.1 μmol L^{-1}) to calibrate the fluorescent resorufin signal. Respiration rate was determined from the rate of decline in O_2 concentration within the respirometry chamber; experimental runs were conducted at concentrations between 250 and 550 nmol/ml O_2 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_{PM}), then glutamate (10 mM) and finally succinate (25 mM) were added to determine the maximal capacity for supporting oxidative phosphorylation (maximum OXPHOS, P_{PMGS}) via complex I and 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 (L_{Omy}). 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_{PMGS}) relative to the Leak respiration state (L_{Omy}; addition of oligomycin). Net phosphorylation efficiency (P_efficiency) was calculated as described by Shama et al. (2016)[35]:

$$\text{P\_efficiency} = 1 - (1/\text{RCR}) \quad (3)$$

The ratio of ROS production to OXPHOS respiration (P_{PMGS}) was calculated as described in Dawson et al.[31]

2.3 | Data analysis

The growth rates of individual fish over the two-week feeding trial were calculated as described in Salin et al.[21]:

$$\text{Specific growth rate} = \left(\text{Ln}(W_f) - \text{Ln}(W_i)\right) \times \left(t^{-1}\right) \times 100 \quad (4)$$

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

Gross growth efficiency was calculated as described in Salin et al. (2019)[21]:

$$\text{Gross growth efficiency} = \left(\text{mass gain} \times \text{day}^{-1}\right) / \left(\text{mass of pellets eaten} \times \text{day}^{-1}\right) \quad (5)$$

Food intake (expressed as % body mass per day) was calculated as described in Salin et al.[21]:

$$\text{Food intake} = 100 \times \left(\text{mass of pellets eaten} \times \text{day}^{-1}\right) / \left(W_i\right) \quad (6)$$

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; http://www.R-project.org/) to determine the relationships between individual variation in mitochondrial physiological parameters (P_efficiency, maximal OXPHOS respiration (P_{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_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_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_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_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_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_efficiency), Leak (L_{Omy}) and maximum OXPHOS (P_{PMGS}) respiration rates, ROS emission rates under P_{PMGS} and the ratio of ROS emission to respiration rates under P_{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_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_{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_{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_{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_{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 ($p < .05$).

states). This increase in respiration rate is an expected effect of temperature on mitochondrial function[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_efficiency ($p = .007$). There were no differences in white muscle mitochondria for either measure of efficiency (RCR, $p = .67$; P_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_{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_{PMGS}), $p = .034$; Table 1).

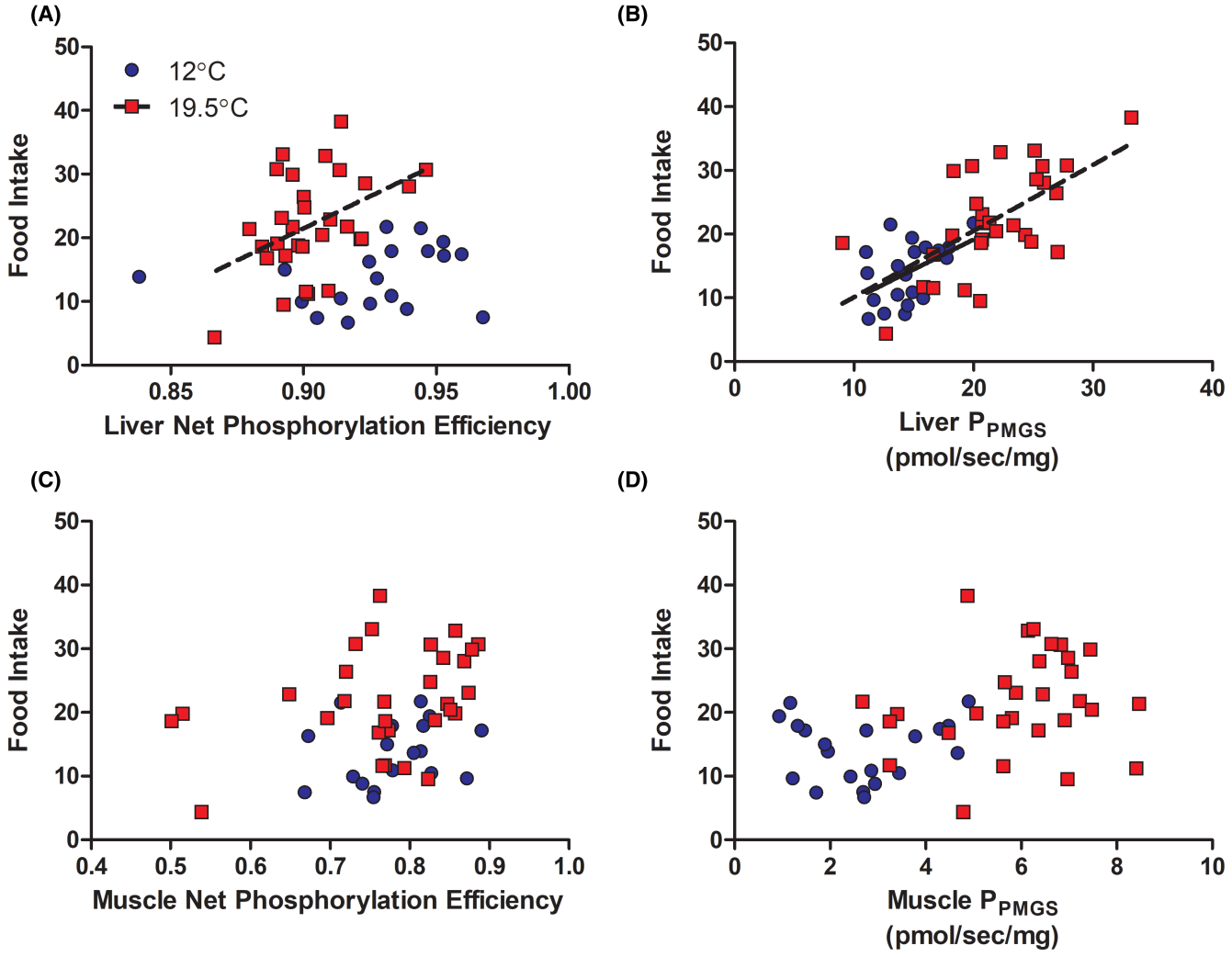

FIGURE 1 Relationship between food intake and (A) net phosphorylation efficiency of liver mitochondria, (B) maximal OXPHOS respiration rate (P_{PMGS}) in liver mitochondria, (C) net phosphorylation efficiency of muscle mitochondria, (D) maximal OXPHOS respiration rate (P_{PMGS}) in muscle mitochondria 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_{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

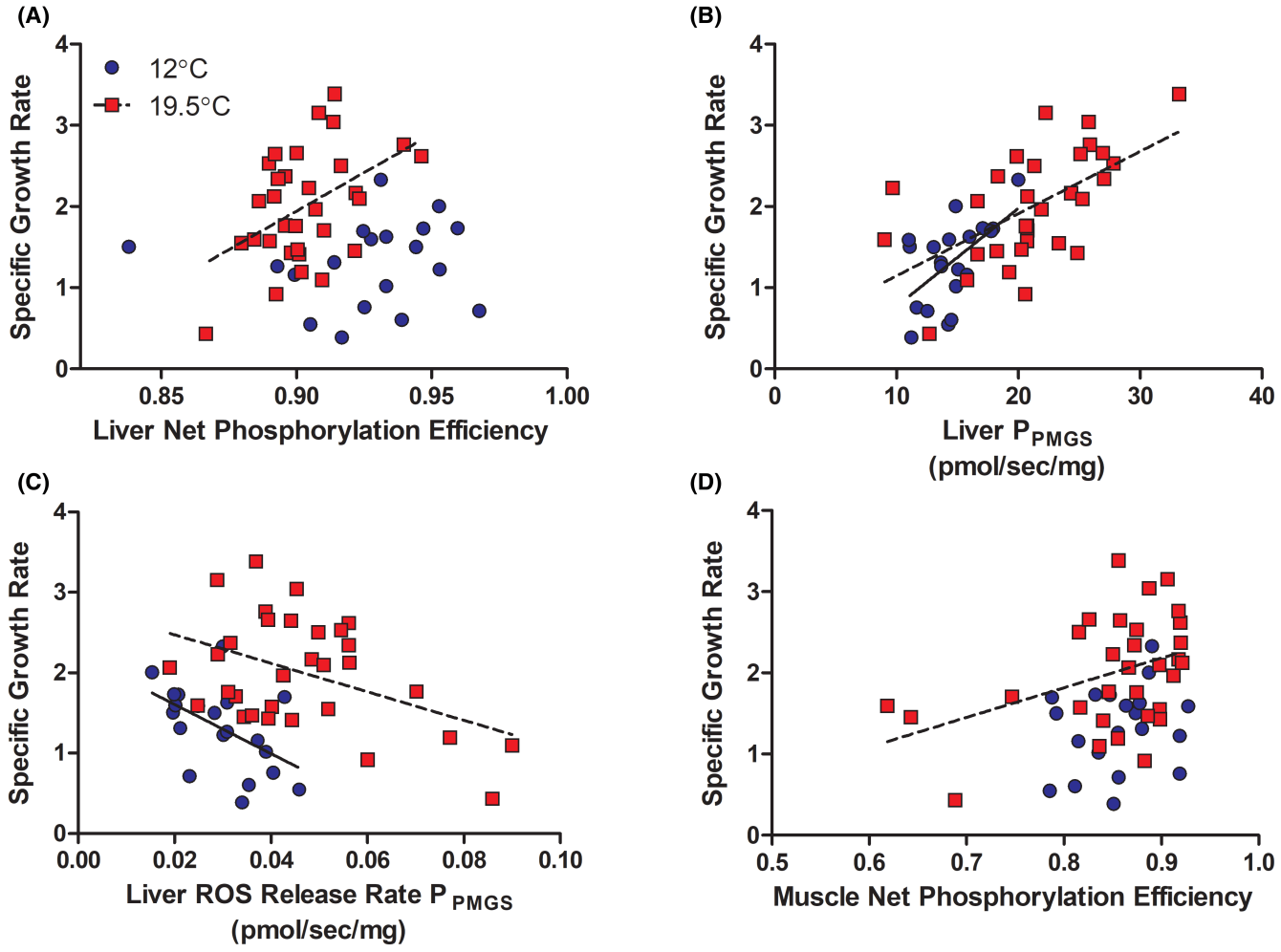

FIGURE 2 Relationship between specific growth rate and (A) net phosphorylation efficiency, (B) maximal OXPHOS respiration rate (P_{PMGS}), (C) ROS release rates (P_{PMGS}) of liver mitochondria, and (D) net phosphorylation efficiency of white muscle mitochondria in juvenile brown trout acclimated to 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

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	−5.499 ± 12.011	
	Temperature[b]	**−39.34 ± 19.175**	**$F_{7,44} = 4.056$, $p = .046$**
	Liver P_efficiency	6.817 ± 14.465	$F_{7,44} = 0.227$, $p = .640$
	Liver OXPHOS (P_{PMGS})	**0.913 ± 0.192**	**$F_{7,44} = 22.156$, $p < .0001$**
	Temperature × Liver P_efficiency	**52.0398 ± 23.3607**	**$F_{7,44} = 4.814$, $p = .031$**
Specific growth rate[a]	Intercept	1.243 ± 3.722	
	Temperature[b]	**−14.702 ± 6.381**	**$F_{8,43} = 5.308$, $p = .026$**
	Liver P_efficiency	−0.758 ± 4.023	$F_{8,43} = 0.035$, $p = .851$
	Liver OXPHOS (P_{PMGS})	**0.0747 ± 0.0174**	**$F_{8,43} = 18.344$, $p = .0001$**
	Liver ROS release (P_{PMGS})	**−11.857 ±5.691**	**$F_{8,43} = 4.343$, $p = .043$**
	Temperature ×Liver P_efficiency	**16.756 ± 7.041**	**$F_{8,43} = 5.664$, $p = .022$**
Growth efficiency[c]	Intercept	0.139 ± 0.015	
	Temperature[b]	**−0.073 ± 0.019**	**$F_{8,27.1} = 15.366$, $p < .001$**
	Muscle ROS release (P_{PMGS})	−5.035 ± 3.668	$F_{8,37.0} = 1.885$, $p = .178$
	Liver ROS release (P_{PMGS})	**−1.133 ± 0.352**	**$F_{8,27.6} = 10.388$, $p = .003$**
	Temperature × Muscle ROS release (P_{PMGS})	**11.976 ± 4.149**	**$F8_{,37.1} = 8.329$, $p = .006$**
	Temperature × Liver ROS release (P_{PMGS})	**0.967 ± 0.401**	**$F_{8,31.3} = 5.803$, $p = .022$**

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 × liver P_{PMGS}) + (temperature × muscle P_{PMGS}) + (temperature × initial body mass) + (temperature ×liver net phosphorylation efficiency) + (temperature × muscle net phosphorylation efficiency) + (temperature × liver ROS) + (temperature × 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 × liver P_{PMGS}) + (temperature × muscle P_{PMGS}) + (temperature × initial body mass) + (temperature × liver net phosphorylation efficiency) + (temperature × muscle net phosphorylation efficiency) + (temperature ×liver ROS) + (temperature × 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 × liver P_{PMGS}) + (temperature × muscle P_{PMGS}) + (temperature × initial body mass) + (temperature × liver net phosphorylation efficiency) + (temperature × muscle net phosphorylation efficiency) + (temperature × liver ROS) + (temperature × muscle ROS) (Table S2).

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.[37] 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.[23] 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

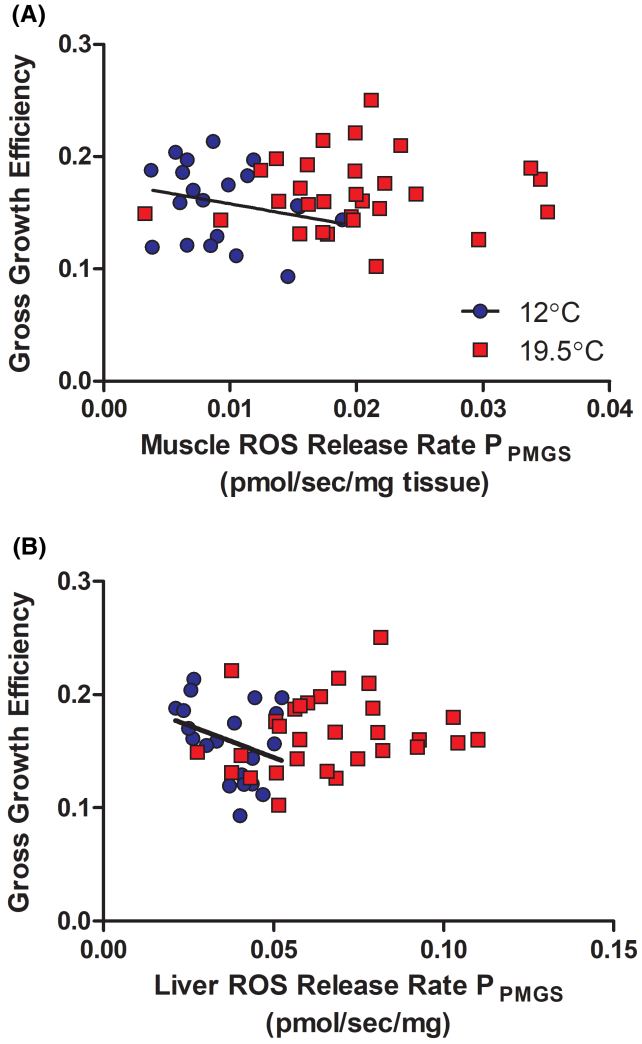

FIGURE 3 Relationship between gross growth efficiency and (A) white muscle ROS release rates (P_{PMGS}) and (B) liver ROS release rates (P_{PMGS}) in juvenile brown trout acclimated to high (19.5°C, $N = 31$) and low (12°C, $N = 20$) temperatures. Lines show significant effects for 12°C (solid line). See Table 2 for statistical analyses

ATP production is decreasing at higher temperatures. The reduced oxygen availability in water at higher temperatures,[38] coupled with increased minimal metabolic demands,[2] 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.[21] 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_{PMGS} 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,[39–42] and liver antioxidant enzymes are often used as a biomarker of environmental contaminants.[43,44] 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).[36] 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.[45–47]

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,[21] 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[48,49]), will individuals with more efficient mitochondria have higher ROS loads leading to an accumulation of ROS-based oxidative damage,[50,51] 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.

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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).

REFERENCES

1. Speers-Roesch B, Norin T. Ecological significance of thermal tolerance and performance in fishes: new insights from integrating field and laboratory approaches. *Funct Ecol*. 2016;30:842-844.
2. Little AG, Loughland I, Seebacher F. What do warming waters mean for fish physiology and fisheries? *J Fish Biol*. 2020;97:328-340.
3. McKenzie DJ, Palstra AP, Planas J, et al. Aerobic swimming in intensive finfish aquaculture: applications for production, mitigation and selection. *Reviews in Aquaculture*. 2020;13:138-155.
4. Seebacher F, White CR, Franklin CE. Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat Clim Chang*. 2015;5:61-66.
5. Gunderson A, Stillman J. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society*. 2015;282:20150401.
6. Comte L, Olden JD. Climatic vulnerability of the world's freshwater and marine fishes. *Nat Clim Chang*. 2017;7:718-723.
7. Pörtner HO, Bock C, Mark FC. Oxygen-and capacity-limited thermal tolerance: bridging ecology and physiology. *J Exp Biol*. 2017;220(15):2685-2696.
8. Jutfelt F, Norin T, Ern R, et al. Oxygen-and capacity-limited thermal tolerance: blurring ecology and physiology. *J Exp Biol*. 2018;221(1):jeb169615.
9. Ohlberger J. Climate warming and ectotherm body size—from individual physiology to community ecology. *Funct Ecol*. 2013;27(4):991-1001.
10. Verberk WC, Atkinson D, Hoefnagel KN, Hirst AG, Horne CR, Siepel H. Shrinking body sizes in response to warming: explanations for the temperature–size rule with special emphasis on the role of oxygen. *Biol Rev*. 2021;96(1):247-268.
11. Secor SM, Carey HV. Integrative physiology of fasting. *Compr Physiol*. 2016;6(2):773-825.
12. Salin K, Auer SK, Anderson GJ, Selman C, Metcalfe NB. Inadequate food intake at high temperatures is related to depressed mitochondrial respiratory capacity. *J Exp Biol*. 2016a;219(9):1356-1362.
13. Gregory TR, Wood CM. Individual variation and interrelationships between swimming performance, growth rate, and feeding in juvenile rainbow trout (*Oncorhynchus mykiss*). *Can J Fish Aquat Sci*. 1998;55(7):1583-1590.
14. Auer SK, Salin K, Rudolf AM, Anderson GJ, Metcalfe NB. The optimal combination of standard metabolic rate and aerobic scope for somatic growth depends on food availability. *Funct Ecol*. 2015;29(4):479-486.
15. Fangue NA, Richards JG, Schulte PM. Do mitochondrial properties explain intraspecific variation in thermal tolerance? *J Exp Biol*. 2009;212:514-522.
16. Blier PU, Lemieux H, Pichaud N. Holding our breath in our modern world: Will mitochondria keep the pace with climate changes? *Can J Zool*. 2014;92:591-601.
17. Iftikar FI, Macdonald JR, Baker DW, Renshaw GMC, Hickey AJR. Could thermal sensitivity of mitochondria determine species distribution in a changing climate? *J Exp Biol*. 2014;217:2348-2357.

18. Chung DJ, Healy TM, McKenzie JL, Chicco AJ, Sparagna GC, Schulte PM. Mitochondria, temperature, and the pace of life. *Integr Comp Biol*. 2018;58(3):578-590.
19. Koch RE, Buchanan KL, Casagrande S, et al. Integrating mitochondrial aerobic metabolism into ecology and evolution. *Trends Ecol Evol*. 2021;4:321-332.
20. Bermejo-Nogales A, Calduch-Giner JA, Pérez-Sánchez J. Unraveling the molecular signatures of oxidative phosphorylation to cope with the nutritionally changing metabolic capabilities of liver and muscle tissues in farmed fish. *PLoS One*. 2015;10:e0122889.
21. Salin K, Villasevil EM, Anderson GJ, et al. Differences in mitochondrial efficiency explain individual variation in growth performance. *Proc R Soc B*. 2019;286(1909):20191466.
22. Thoral E, Roussel D, Chinopoulos C, Teulier L, Salin K. Low oxygen levels can help to prevent the detrimental effect of acute warming on mitochondrial efficiency in fish. *Biol Let*. 2021;17(2):20200759.
23. Salin K, Auer SK, Rudolf AM, Anderson GJ, Selman C, Metcalfe NB. Variation in metabolic rate among individuals is related to tissue-specific differences in mitochondrial leak respiration. *Physiol Biochem Zool*. 2016b;89(6):511-523.
24. Korshunov SS, Skulachev VP, Starkov AA. High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Lett*. 1997;416:15-18.
25. Miwa S, Brand MD. Mitochondrial matrix reactive oxygen species production is very sensitive to mild uncoupling. *Biochem Soc Trans*. 2003;31:1300-1301.
26. Brand MD. Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. *Free Radic Biol Med*. 2016;100:14-31.
27. Salin K, Villasevil EM, Anderson GJ, et al. Decreased mitochondrial metabolic requirements in fasting animals carry an oxidative cost. *Funct Ecol*. 2018;32(9):2149-2157.
28. Pascual P, Pedrajas JR, Toribio F, López-Barea J, Peinado J. Effect of food deprivation on oxidative stress biomarkers in fish (*Sparus aurata*). *Chem Biol Interact*. 2003;145:191-199.
29. Geiger S, Kauffmann M, Le Maho Y, Robin J-P, Criscuolo F. Of the importance of metabolic phases in the understanding of oxidative stress in prolonged fasting and refeeding. *Physiol Biochem Zool*. 2012;85:415-420.
30. Elliott JM. The energetics of feeding, metabolism and growth of brown trout (*Salmo trutta L.*) in relation to body weight, water temperature and ration size. *J Anim Ecol*. 1976;923-948.
31. Dawson NJ, Lyons SA, Henry DA, Scott GR. Effects of chronic hypoxia on diaphragm function in deer mice native to high altitude. *Acta Physiol*. 2018;223(1):e13030.
32. Závorka L, Crespel A, Dawson NJ, Papatheodoulou M, Killen SS, Kainz MJ. Climate change induced deprivation of dietary essential fatty acids can reduce growth and mitochondrial efficiency of wild juvenile salmon. *Funct Ecol*. 2021;35:1960-1971.
33. Rasmussen HN, Rasmussen UF. Small scale preparation of skeletal muscle mitochondria, criteria of integrity, and assays with reference to tissue function. In: FN Gellerich, S Zierz, eds. *Detection of Mitochondrial Diseases*. Springer; 1997:55-60.
34. Kuznetsov AV, Schneeberger S, Seiler R, et al. Mitochondrial defects and heterogeneous cytochrome c release after cardiac cold ischemia and reperfusion. *Am J Physiol-Heart Circul Physiol*. 2004;286(5):H1633-H1641.
35. Shama LN, Mark FC, Strobel A, Lokmer A, John U, Mathias Wegner K. Transgenerational effects persist down the maternal line in marine sticklebacks: gene expression matches physiology in a warming ocean. *Evol Appl*. 2016;9:1096-1111.
36. Gerber L, Clow KA, Mark FC, Gamperl AK. Improved mitochondrial function in salmon (*Salmo salar*) following high temperature acclimation suggests that there are cracks in the proverbial "ceiling". *Sci Rep*. 2020;10(1):1-12.
37. Brusle J, Anadon GG. The structure and function of fish liver. *Fish Morphol*. 1996;76:545-551.
38. Harris KR, Woolf LA. Pressure and temperature dependence of the self diffusion coefficient of water and oxygen-18 water. *J Chem Soc Faraday Trans 1*. 1980;76:377-385.
39. Crespo M, Solé M. The use of juvenile *Solea solea* as sentinel in the marine platform of the Ebre Delta: in vitro interaction of emerging contaminants with the liver detoxification system. *Environ Sci Pollut Res*. 2016;23(19):19229-19236.
40. Siscar R, Koenig S, Torreblanca A, Solé M. The role of metallothionein and selenium in metal detoxification in the liver of deep-sea fish from the NW Mediterranean Sea. *Sci Total Environ*. 2014;466:898-905.
41. Wang L, Liang XF, Liao WQ, Lei LM, Han BP. Structural and functional characterization of microcystin detoxification-related liver genes in a phytoplanktivorous fish, Nile tilapia (*Oreochromis niloticus*). *Comp Biochem Physiol C: Toxicol Pharmacol*. 2006;144(3):216-227.
42. Monserrat JM, Lima JV, Ferreira JLR, et al. Modulation of antioxidant and detoxification responses mediated by lipoic acid in the fish *Corydoras paleatus* (*Callychthyidae*). *Comp Biochem Physiol C: Toxicol Pharmacol*. 2008;148(3):287-292.
43. Lenartova V, Holovska K, Rafael Pedrajas J, et al. Antioxidant and detoxifying fish enzymes as biomarkers of river pollution. *Biomarkers*. 1997;2(4):247-252.
44. Aceto A, Amicarelli F, Sacchetta P, et al. Developmental aspects of detoxifying enzymes in fish (*Salmo iridaeus*). *Free Radical Res*. 1994;21(5):285-294.
45. Andrade FH, Reid MB, Allen DG, Westerblad H. Effect of hydrogen peroxide and dithiothreitol on contractile function of single skeletal muscle fibres from the mouse. *J Physiol*. 1998;509(2):565-575.
46. Radak Z, Taylor AW, Ohno H, Goto S. Adaptation to exercise-induced oxidative stress: from muscle to brain. *Exer Immunol Rev*. 2001;7:90-107.
47. Gram M, Vigelsø A, Yokota T, Helge JW, Dela F, Hey-Mogensen M. Skeletal muscle mitochondrial H_2O_2 emission increases with immobilization and decreases after aerobic training in young and older men. *J Physiol*. 2015;593(17):4011-4027.
48. Metcalfe NB, Monaghan P. Compensation for a bad start: grow now, pay later? *Trends Ecol Evol*. 2001;16(5):254-260.
49. Metcalfe NB, Monaghan P. Growth versus lifespan: perspectives from evolutionary ecology. *Exp Gerontol*. 2003;38(9):935-940.
50. Monaghan P, Metcalfe NB, Torres R. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecol Lett*. 2009;12(1):75-92.

51. Metcalfe NB, Alonso-Alvarez C. Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. *Funct Ecol*. 2010;24(5):984-996.

SUPPORTING INFORMATION

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

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