PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

Cite this article: Salin K, Villasevil EM, Anderson GJ, Lamarre SG, Melanson CA, McCarthy I, Selman C, Metcalfe NB. 2019 Differences in mitochondrial efficiency explain individual variation in growth performance. *Proc. R. Soc. B* **286**: 20191466. http://dx.doi.org/10.1098/rspb.2019.1466

Received: 21 June 2019
Accepted: 1 August 2019

Subject Category:
Development and physiology

Subject Areas:
ecology, physiology, evolution

Keywords:
ATP/O ratio, brown trout, energy metabolism, intraspecific, mitochondrial plasticity, protein synthesis

Author for correspondence:
Karine Salin
e-mail: salin.karine@gmail.com

[†] Present address: Ifremer Laboratory of Environmental Marine Sciences, 29280 Plouzané, France.

Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.4611455.

THE ROYAL SOCIETY
PUBLISHING

Differences in mitochondrial efficiency explain individual variation in growth performance

Karine Salin[1,†], Eugenia M. Villasevil[1], Graeme J. Anderson[1], Simon G. Lamarre[2], Chloé A. Melanson[2], Ian McCarthy[3], Colin Selman[1] and Neil B. Metcalfe[1]

[1]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow G12 8QQ, UK
[2]Département de Biologie, Université de Moncton, Moncton, New Brunswick, Canada E1A 3E9
[3]School of Ocean Sciences, Bangor University, Menai Bridge LL59 5AB, UK

KS, 0000-0002-3368-9639; NBM, 0000-0002-1970-9349

The physiological causes of intraspecific differences in fitness components such as growth rate are currently a source of debate. It has been suggested that differences in energy metabolism may drive variation in growth, but it remains unclear whether covariation between growth rates and energy metabolism is: (i) a result of certain individuals acquiring and consequently allocating more resources to growth, and/or is (ii) determined by variation in the efficiency with which those resources are transformed into growth. Studies of individually housed animals under standardized nutritional conditions can help shed light on this debate. Here we quantify individual variation in metabolic efficiency in terms of the amount of adenosine triphosphate (ATP) generated per molecule of oxygen consumed by liver and muscle mitochondria and examine its effects, both on the rate of protein synthesis within these tissues and on the rate of whole-body growth of individually fed juvenile brown trout (*Salmo trutta*) receiving either a high or low food ration. As expected, fish on the high ration on average gained more in body mass and protein content than those maintained on the low ration. Yet, growth performance varied more than 10-fold among individuals on the same ration, resulting in some fish on low rations growing faster than others on the high ration. This variation in growth for a given ration was related to individual differences in mitochondrial properties: a high whole-body growth performance was associated with high mitochondrial efficiency of ATP production in the liver. Our results show for the first time, to our knowledge, that among-individual variation in the efficiency with which substrates are converted into ATP can help explain marked variation in growth performance, independent of food intake. This study highlights the existence of inter-individual differences in mitochondrial efficiency and its potential importance in explaining intraspecific variation in whole-animal performance.

1. Introduction

Individual animals may grow at widely differing rates despite living under the same conditions—a finding that has been documented across a broad range of taxa (reviewed in [1,2]). This phenomenon is often interpreted in terms of variation in individual quality. For instance, individuals that grow faster typically reach maturity more quickly and can have higher fecundity than slower growing individuals, suggesting direct fitness consequences of growth rate [3,4]. However, the physiological processes underlying this among-individual variation in growth rate are currently poorly understood.

Faster growth can obviously be achieved by increasing food intake. Individuals with a high rate of food intake grow faster compared to individuals that have a lower rate of resource intake, because high amounts of food intake can lead to an increased rate of resource allocation to energetically costly processes, such as biomass production and, in turn, growth. However, variation in growth rate may persist even when food intake is standardized. For example, individual fish fed to satiation and consuming a similar amount of food exhibited threefold differences in growth performance [5]. Similarly, fivefold differences in the rate of growth have been shown among fish consuming an identical amount of food [6]. This suggests that variation in growth may be, at least partly, attributed to variation in the efficiency of resource utilization and its allocation to biomass production. Yet surprisingly little research has investigated the possible mechanisms that might underlie this variation in metabolic efficiency and thus growth performance [7].

Variation in the efficiency with which food is converted to energy is thought to play an important role in the association between food intake and animal growth [7–9]. Energy derived from nutrients becomes usable for cellular processes only following the transformation into high-energy molecules of adenosine triphosphate (ATP). ATP is the principal energy source for most cellular functions, such as DNA, RNA and protein synthesis (and hence biomass production). The main sites of energy conversion are the mitochondria, which provide over 90% of a cell's ATP [10]. Mitochondrial ATP is produced via oxidative phosphorylation, a process through which energy substrates are oxidized to generate a proton gradient that drives the phosphorylation of ADP to ATP. Although ATP production depends on the rate of substrate oxidation, the number of ATP molecules produced for each molecule of oxygen and energy substrate (i.e. pyruvate, glutamate, acetyl-CoA, etc.) consumed by the mitochondria can vary [11]. A proportion of the energy that is generated from substrate oxidation is dissipated through proton leakage across the inner mitochondrial membrane and this leakage might decrease the energy available to produce ATP [12]. The amount of energy dissipated in the mitochondrial proton leak varies among individuals [13,14] and this variation is known to correlate with the animal performance [15,16]. This raises the possibility that variation in growth among individuals could involve differences in the efficiency through which mitochondria produce ATP.

Mitochondrial efficiency can be quantified through the measurement of the ATP/O ratio; that is the ratio in the amount of ATP generated per unit of oxygen consumed [17]. Thus, the higher this ratio, the more efficiently an animal converts its metabolic substrates into ATP, with the ATP then available for energy-demanding cellular processes such as protein synthesis and biomass production [18]. A number of studies have found positive links between mean growth rate and mean mitochondrial efficiency when comparing among treatment groups, populations or selection lines [9,19–23], but until now there has, to our knowledge, been no assessment of whether mitochondrial efficiency could explain variation in growth rate among individual animals maintained with the same food intake.

In this study, we tested, for the first time to our knowledge, whether individual variation in growth performance—measured both as the rate of whole-body gain in mass and as the rate of protein synthesis—was related to among-individual variation in mitochondrial efficiency. To test this hypothesis, we assessed the relationships between ATP/O ratio, fractional rate of protein synthesis and growth performance (growth rate, growth efficiency and protein gain) among individually housed brown trout (*Salmo trutta*) of the same age and maintained under standardized conditions. In order to standardize their food intake, fish were fed on individual limited rations to ensure that differences in growth performance could be attributed to mitochondrial efficiency differences. We chose juvenile brown trout as our study organism because larger body size in brown trout is a major determinant of fitness, with fast growth resulting in increased survival [24] and larger body size being linked to higher fecundity [25]. We analysed mitochondrial properties and protein synthesis in the liver and the white muscle because the physiological properties of these tissues are known to influence growth performance [16,26]. We predicted positive inter-individual correlations among mitochondrial efficiency, protein synthesis and growth performance.

2. Material and methods

(a) Experimental animals

Brown trout fry was moved from the hatchery (Howietoun, UK) to the University of Glasgow in June 2015. The fish were then kept in a communal tank and maintained under a 12 L : 12 D photoperiod at 12°C and fed daily in excess with trout pellet food (EWOS, West Lothian, UK). In September 2016, fish ($n = 60$) were transferred to individual compartments within a stream tank system that allowed individual daily feeding while maintaining fish under the same water quality conditions. Each individual compartment contained a small shelter (a section of opaque plastic pipe).

The fish were first acclimated for two weeks in their individual compartments, during which they were hand-fed daily to excess on the same trout pellets. Fish were then fasted for 22 h and briefly anaesthetized (50 ml l^{-1} benzocaine in water) for measurement of body mass (±0.001 g) to allow calculation of caloric intake and thereby food rations (as number of pellets). For the next 5–10 weeks (see below), the fish were fed once daily on an intermediate ration of pellets (presumed sufficient for growth but less than a maximal rate of intake) using an equation from Elliott [27]; this allowed calculation of individual-specific rations in calories as a function of the fish's body mass (W) in grams and water temperature (T) of 12°C as follows:

$$\text{intermediate ration} = 24.062 \times W^{0.737} \times \exp(0.105 \times T).$$

Fish were fed their ration in the early morning; all fish consumed their entire daily ration within 2 h. Body mass was measured every two weeks, and food rations were recalculated to adjust for gains in mass. Fish were fasted for 22 h before each body mass measurement, and on return to their compartment were fed 2 h later than usual to allow time to recover from the anaesthetic and to ensure they ate the ration. All fish consumed their entire daily ration and gained mass during this acclimation period.

(b) Diet treatment and growth measurements

Following this period of acclimation to an intermediate diet, fish were switched to the final diet treatment for 14 days. This duration was chosen because it limited the extent of mitochondrial turn-over that would occur over the growth period but was sufficient to detect differences in the rate of growth between individuals [28]. Because only two individuals per day could be analysed for their mitochondrial function at the end of the experiment, the start of the diet treatment was staggered over a five-week period (so that the preceding acclimation period varied between 5 and 10 weeks). Two fish per day (which would subsequently be processed together 14 days later) were thus randomly allocated to the treatments: one fish had its ration increased to 150% of the intermediate ration (high ration,

$n = 30$) and the other had its ration decreased to 50% of the intermediate ration (low ration, $n = 30$). The low ration was estimated to provide sufficient energy to cover maintenance requirements and relatively slow growth [27], while the high ration approximated the maximal rate of food intake of juvenile brown trout [27]. Body mass ranged from 3.61 to 15.48 g across individuals at the start of the experiment but did not differ between fish subsequently assigned to the two food treatments (high ration: 8.15 ± 0.49 g, low ration: 8.18 ± 0.48 g, *t*-test: $t_{58} = -0.041$, $p = 0.967$). Body mass was re-measured (as above) at day 7 of the diet treatment, and rations were recalculated to adjust for growth. All but one fish consumed their entire daily ration within 2 h during the experimental period; this fish was removed from all analyses so giving a final sample size of 59 fish (high food: $n = 29$; low food: $n = 30$).

Growth rate and growth efficiency were simultaneously estimated over a 7-day period starting at day 7 of the experimental treatment (termed the initial fish body mass (BM) in the following equation) and ending at day 14 (final fish BM). Specific growth rate (% day^{-1}) was defined as:

$$\text{specific growth rate} = \frac{\ln(\text{final BM}) - \ln(\text{initial BM})}{\text{days elapsed}} \times 100.$$

Daily food intake was calculated from the daily food ration and was expressed in terms of pellet mass. Growth efficiency (mg gain in body mass mg^{-1} food eaten) was measured for each fish as:

$$\text{growth efficiency} = \frac{\text{gain in BM day}^{-1}}{\text{mass of pellets eaten day}^{-1}}.$$

At the end of the food treatment period, fractional rates of protein synthesis and mitochondrial properties were measured in the fish following protocols described below.

(c) Estimate of gain in whole-body protein

The relationship between whole-body protein content and body mass of fish reared under intermediate, low and high rations was used to estimate the protein content of each fish at the start and at the end of the diet treatment and thereby estimate the gain in protein content over the treatment period. Specifically, we first determined the relationship between the body mass of a fish and its whole-body protein content (electronic supplementary material, figure S1), using a separate group of brown trout of the same age and size (see the electronic supplementary material for full details in section ‘Whole-body protein content’).

The initial whole-body protein content (initial BP) of each experimental fish was therefore estimated from its body mass at the start of the food treatment, using the calibration regression for fish on the intermediate ration. The final whole-body protein content (final BP) of each experimental fish was likewise estimated from its body mass at the end of the food treatment, using the appropriate equation for its diet treatment. Specific protein gain rate (% day^{-1}) was then defined as:

$$\text{specific protein gain} = \frac{\ln(\text{final BP}) - \ln(\text{initial BP})}{\text{days elapsed}} \times 100.$$

(d) Measurement of the fractional rate of protein synthesis

The percentage of the protein mass synthesized per day—the fractional rate of protein synthesis—was measured using the flooding dose assay [29], modified for using stable isotope tracer, the ring-D5-phenylalanine (D_5-Phe) [30]. In short, the ratios of the amount of D_5-Phe relative to the amount of total phenylalanine (total Phe equal to D_5-Phe plus its natural version) in both the protein pool and the free pool of amino acids allow calculation of the fractional rate of protein synthesis. The assay was first validated for brown trout of this age and size by conducting a preliminary time-course experiment (electronic supplementary material). From this validation experiment, we determined that a D_5-Phe incubation period of approximately 60 min was an appropriate incorporation duration.

For the main experiment, the fish were fasted for 21 h before being injected into the peritoneum with the D_5-Phe solution. Each fish was then immediately placed in an individual tank containing 2 l of aerated water for a period of approximately 1 h (mean ± s.e.: 1 h05 min ± 0h00 min) without food and in darkness. The fish were then culled and their livers were immediately dissected, weighed and rinsed with distilled water. A subsample of liver was weighed and kept in ice-cold respirometry buffer (0.1 mM EGTA, 15 µM EDTA, 1 mM $MgCl_2$, 20 mM Taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM D-sucrose, 60 mM lactobionic acid, 1 g l^{-1} bovine serum albumin essentially fatty acid-free, pH 7.2 with KOH) for subsequent measurement of mitochondrial properties (see below). A second aliquot of the liver for the measurement of protein synthesis was weighed and immediately flash-frozen in liquid nitrogen and stored at −70°C until further analysis. Likewise, two samples of white muscle were taken dorsally to the lateral line (to avoid contamination with red fibres) and just behind the dorsal fin. One aliquot was collected from one side of the fish and kept in respirometry buffer while the other aliquot was collected from the other side and immediately flash-frozen. After extraction and quantification of the phenylalanine isotopes in both the free amino acid pool and in the protein pool (details in the electronic supplementary material), the fractional rate of protein synthesis (Ks in % day^{-1}) was calculated as:

$$\text{Ks} = \frac{24}{t} \times \frac{(\text{D}_5\text{Phe}/\text{total Phe}) \text{ in protein amino acid}}{(\text{D}_5\text{Phe}/\text{total Phe}) \text{ in free amino acid}} \times 100.$$

where t is the actual duration of D_5-Phe exposure in hours.

(e) Measurement of mitochondrial properties

Because only two samples could be run simultaneously to measure mitochondrial properties, liver samples of the two individuals in a processing batch were first homogenized as in [15,16] and assessed for mitochondrial function, while the subsample of white muscle was preserved in respirometry buffer on ice for the subsequent run.

Oxygen and magnesium green fluorescence signals were detected simultaneously using two respirometry chambers equipped with fluorescent sensors and recorded using DatLab software (Oroboros Instruments, Innsbruck Austria). Tissue homogenate from each fish was added to one of the two measurement chambers immediately following preparation. Mitochondrial efficiency was measured as in Salin *et al.* [31]. Briefly, we used a protocol for estimating the ATP/O ratio that simultaneously measures both oxygen consumption and ATP production on the same sample. Cytochrome *c* oxidase (COX) respiration was then measured to allow standardization of the mitochondrial density of the tissues [32]. The rate of oxygen consumption simultaneously to ATP production was assessed by adding saturating ADP to the chamber containing complex I and II substrates. COX activity was measured after addition of ascorbate and *N,N,N′,N′*-Tetramethyl-*p*-phenylenediamine dihydrochloride. The muscle trial was identical to the liver trial but adenylate kinase inhibitor was added to the measurement chamber with the subsample of muscle that was kept on ice (see the electronic supplementary material for full details of the protocol).

Rates of mass-specific oxygen consumption and ATP production at each step of the protocol were averaged over

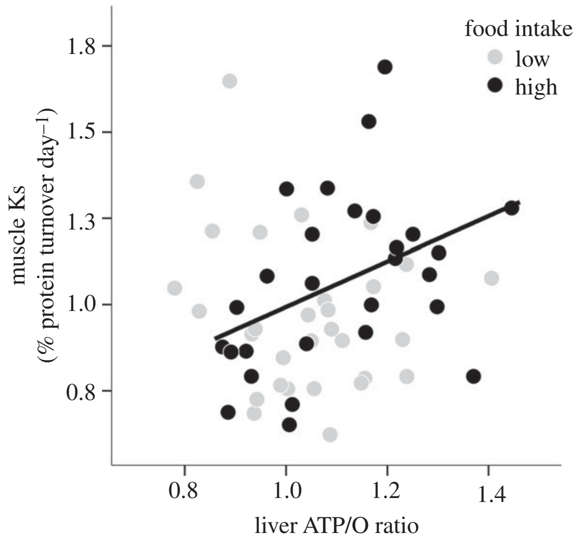

Figure 1. Relationship between the fractional rate of protein synthesis (Ks) in the muscle and mitochondrial efficiency (ATP/O ratio) in the liver of juvenile brown trout at low versus high food intake. Continuous lines show significant effect. $n = 28$–30 fish per food level. See table 1 for statistical analyses.

30–60 s of stabilization. Fluxes of O_2 and ATP were expressed in pmoles s^{-1} mg^{-1} wet weight of tissue. The ATP/O ratio was calculated as the ratio of corrected ATP production to double the rate of O_2 consumption at the time that the ATP was being produced.

(f) Statistical analysis

We first used correlation analysis to test whether physiological parameters (mitochondrial efficiency (ATP/O ratio), mitochondrial density (COX activity) and fractional rate of protein synthesis (Ks)) were correlated between the liver and white muscle within the same fish. We then used linear mixed models (LMMs) to determine the links between mitochondrial efficiency of the liver and/or muscle and the fractional rate of protein synthesis for different rates of food intake. The models included Ks of liver or muscle as the dependent variable, ATP/O ratio of liver and muscle as continuous predictors, and the food intake (high or low) as a fixed factor, and two-way interactions between food intake and covariates. To control for effects of mitochondrial density on the fractional rate of protein synthesis, the models included COX activity of the liver and muscle as a covariate and in two-way interactions with food intake, with Ks as the dependent variable. Processing batch was included as a random effect to control for the order in which fish were processed. Preliminary analyses showed that the fractional rate of protein synthesis was not affected by the duration of D_5-Phe exposure or the mass of sample used for the extraction of the phenylalanine isotopes, so exposure duration and mass of sample were not included as covariates in the final models. We finally tested whether the degree of mitochondrial efficiency and the fractional rate of protein synthesis of the liver and/or the muscle explained individual variation in growth performance using a linear mixed model approach. The models included the growth performance (specific growth rate, growth efficiency and specific protein gain) as dependent variables, and ATP/O ratio and Ks of liver and muscle as continuous predictors, the food intake as a fixed factor, with processing batch as a random factor. To control for effects of mitochondrial density on growth performance, COX activity of the liver or muscle were included as a covariate in the models with specific growth rate, growth efficiency and specific protein gain as the dependent variable. These models also included two-way interactions between covariates and food regime. To control for effects of initial body size on growth performance, initial body mass was included as a covariate in the models with specific growth rate or growth efficiency as the dependent variable, while the initial estimate for whole-body protein content was included as a covariate in the model for specific protein gain. All models were simplified by removing non-significant terms in a backward deletion procedure, starting with two-way interactions; significance was tested when terms were dropped from the model. All statistical analyses were performed in IBM SPSS Statistics 21 (Chicago, IL). Data are presented as means ± s.e., and the significance level was set to $p < 0.05$.

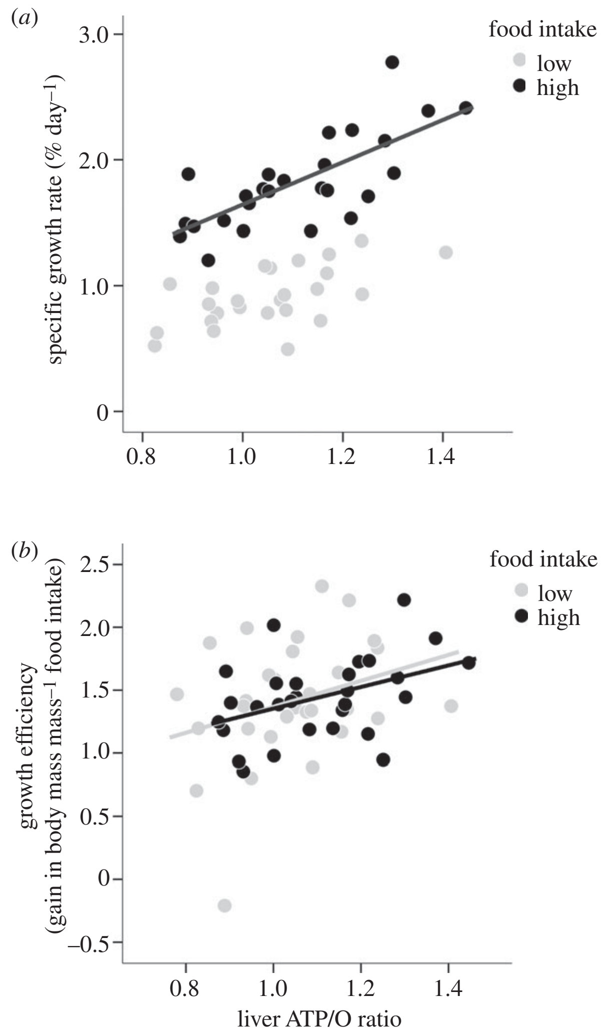

Figure 2. Relationships between indices of growth performance and mitochondrial efficiency (ATP/O ratio) in juvenile brown trout at low versus high food levels. (*a*) Specific growth rate in relation to liver ATP/O ratio, and (*b*) growth efficiency in relation to liver ATP/O ratio. Continuous lines show significant effects. $n = 29$–30 fish per food level. See table 2 for statistical analyses. (*a*) Plotted are partial residuals of specific growth rate for fish at high food ration evaluated at mean initial body mass = 9.59 g. (*b*) Plotted are partial residuals of growth efficiency evaluated at mean initial body mass = 9.02 mg.

3. Results

The mitochondrial efficiency (ATP/O ratio) showed significant inter-individual variation, varying at least twofold for each tissue across individuals having the same food intake (electronic supplementary material, table S1). The fractional rate of protein synthesis Ks differed up to two- or fivefold in liver and muscle, respectively, among individuals with the same food intake (electronic supplementary material, table S1). There was no correlation between the physiological

Table 1. Results from linear mixed model analysis of the fractional rate of protein synthesis (Ks) in the muscle of a brown trout as a function of its food intake and the properties (ATP/O ratio and cytochrome *c* oxidase, COX activity) of mitochondria in its muscle and liver. (Processing batch was included as a random effect to control for the order in which fish were processed. Non-significant terms were excluded from the final analysis. Bold denotes significant results.)

dependant variable	source of variation	parameter estimate ± s.e.	F	d.f.	p-value
muscle Ks[b]	intercept	−0.00 ± 0.41			
	food intake[a]	**0.88 ± 0.42**	**4.38**	**1, 39.71**	**0.043**
	liver COX activity	0.00 ± 0.01	0.04	1, 46.95	0.837
	muscle COX activity	**0.03 ± 0.01**	**5.25**	**1, 36.56**	**0.028**
	liver ATP/O ratio	0.66 ± 0.23	1.30	1, 30.99	0.262
	muscle ATP/O ratio	−0.03 ± 0.03	1.17	1, 26.98	0.289
	food intake[a] × liver ATP/O ratio	**−0.92 ± 0.39**	**5.58**	**1, 40.41**	**0.023**

[a] Food intake: two-level fixed factor (low and high food intake).

[b] Full model: muscle Ks = food intake + liver COX activity + muscle COX activity + liver ATP/O ratio + muscle ATP/O ratio + food intake × liver ATP/O ratio + food intake × liver COX activity + food intake × muscle COX activity + food intake × muscle ATP/O ratio.

traits (ATP/O ratio and Ks) of the liver and muscle from the same fish (electronic supplementary material, table S2).

The fractional rate of muscle protein synthesis Ks in a fish depended on the ATP/O ratio of its liver mitochondria, although this effect depended on food intake (liver ATP/O by food intake interaction, table 1). While muscle Ks was positively related to the ATP/O ratio in the liver mitochondria of fish with the high food ration ($t_{36} = 2.80$, $p = 0.008$), there was no such relationship in fish receiving a low food ration ($t_{36} = -0.92$, $p = 0.362$; figure 1). Among-individual variation in the fractional rate of protein synthesis Ks in the liver was not explained by the mitochondrial efficiency in either liver or muscle (LMM, $p > 0.05$).

Not surprisingly, food intake had a positive effect on specific growth rates, with fish on average having a specific growth rate threefold higher at the high compared to the low ration (electronic supplementary material, table S1). However, individuals from the same food treatment varied considerably in their specific growth rate, with the fastest growing fish in the low ration exceeding the growth of some fish on the high ration (figure 2*a*; low food intake: −6.00 to 110.57 mg day^{-1}; high food intake: 68.86–394.43 mg day^{-1}). This individual variation in growth rate was partially explained by differences in liver mitochondrial efficiency, although the effect depended on food intake (liver ATP/O by food treatment interaction; table 2). The specific growth rate of fish receiving high rations was strongly and positively linked to the ATP/O ratio in their liver mitochondria ($t_{41} = 4.46$, $p < 0.001$; figure 2*a*), whereas the trend was not significant when food intake was low ($t_{41} = 0.33$, $p = 0.745$). Regardless of the food intake, the specific growth rate of a fish was strongly but negatively linked to the Ks in its muscle after controlling for liver ATP/O (table 2). Specific growth rates under either ration were unrelated to the ATP/O ratio in muscle mitochondria, or to the Ks in the liver (table 2).

Growth efficiency varied among individuals from −0.13 to 2.23 gain in body mass per mass of food eaten but did not differ between low and high food fish (electronic supplementary material, table S1). Regardless of their food intake, individuals that had the higher ATP/O ratio in the liver had the highest growth efficiency (table 2, figure 2*b*).

The rate of protein gain of the trout also differed considerably among individuals, ranging from −1.98 to 17.74 mg day^{-1} for fish eating the low ration and from −0.21 to 60.79 mg day^{-1} for fish on the high ration. Individuals that had a higher ATP/O ratio in their liver mitochondria and a lower Ks in their muscle had a faster specific gain in protein mass (table 2). The specific rate of protein gain was not related to ATP/O ratio in the muscle mitochondria nor to Ks in the liver (table 2).

4. Discussion

While the general trend was for growth performance to increase when food intake was higher, individuals exhibited markedly differing growth performance even when having identical food intake. This variation in growth was related to mitochondrial function: individuals that were more efficient at producing ATP within their liver mitochondria grew faster, more efficiently and accumulated more protein than those individuals with less efficient mitochondria. Individuals that had a higher liver mitochondrial efficiency under high food levels had a faster rate of protein synthesis in their muscle. However, these differences in protein synthesis had an effect on growth performance in the complete opposite direction to our initial prediction that 'protein synthesis promotes growth'. In summary, our study shows for the first time, to our knowledge, that under conditions of fixed food intake, the mitochondrial efficiency of an individual animal can determine whether it grows fast or slowly.

Individual variation in growth performance is likely to be a complex, integrative characteristic influenced by several physiological and behavioural traits. Because individual differences in growth rate covary with behaviours that increase feeding rates [33], only studies of animals with controlled food intakes can shed light on the physiological drivers of growth differences. Food intake in our experiment was standardized, revealing that growth of fish under the same ration could vary more than threefold among individuals. Consequently, some fish on the low ration treatment were actually faster growing than others on the high ration treatment that were consuming three times as much food. While it has previously been shown that increased mitochondrial efficiency promotes fitness-related traits (physical performance [34], growth performance [9,21–23,35], reproductive output [36] and ageing [9,14,36,37]), here we demonstrate that this relationship can even occur when animals are experiencing similar rates of food intake. As well as varying among

Table 2. Results from linear mixed model analyses of indices of growth performance in individual brown trout as a function of their initial mass, their liver and muscle mitochondrial density (COX activity), food intake, liver and muscle mitochondrial efficiency (ATP/O ratio) and fractional rates of protein synthesis (Ks). (Processing batch was included as a random effect to control for the order in which fish were processed. Non-significant terms were excluded from the final analysis. Bold denotes significant results.)

dependant variable	source of variation	parameter estimate ± s.e.	*F*	d.f.	*p*-value
specific growth rate[a]	intercept	−0.38 ± 0.59			
	initial body mass	**0.05 ± 0.02**	**9.69**	**1, 41**	**0.003**
	liver COX activity	−0.01 ± 0.01	0.71	1, 41	0.403
	muscle COX activity	**0.06 ± 0.02**	**8.27**	**1, 41**	**0.006**
	food intake[a]	0.55 ± 0.59	0.87	1, 41	0.355
	liver ATP/O ratio	1.61 ± 0.36	11.7	1, 41	0.001
	muscle ATP/O ratio	−0.02 ± 0.04	0.18	1, 41	0.671
	liver Ks	−0.01 ± 0.02	0.30	1, 41	0.586
	muscle Ks	**−0.54 ± 0.20**	**7.58**	**1, 41**	**0.009**
	food intake[a] × liver ATP/O ratio	**−1.49** ± 0.54	**7.56**	1, 41	**0.009**
growth efficiency[c]	intercept	0.13 ± 0.41			
	initial body mass	**0.06 ± 0.02**	**10.8**	**1, 48**	**0.002**
	liver ATP/O ratio	**0.72 ± 0.33**	**4.87**	**1, 48**	**0.032**
specific protein gain[d]	intercept	−3.03 ± 0.74			
	initial protein mass	0.00 ± 0.00	81.3	1, 31.25	<0.001
	liver COX activity	0.02 ± 0.01	2.80	1, 39.94	0.102
	muscle COX activity	0.09 ± 0.03	0.11	1, 33.93	0.299
	food intake[a]	2.15 ± 0.85	6.34	1, 33.81	0.017
	liver ATP/O ratio	**1.04 ± 0.29**	**13.0**	**1, 30.84**	**<0.001**
	muscle ATP/O ratio	0.02 ± 0.05	0.16	1, 18.86	0.690
	liver Ks	0.01 ± 0.02	0.28	1, 35.40	0.601
	muscle Ks	**−0.51 ± 0.24**	**4.44**	**1, 37.94**	**0.042**
	food intake[a] × initial protein mass	**−0.00 ± 0.00**	**29.4**	**1, 19.30**	**<0.001**
	food intake[a] × muscle COX activity	**−0.13 ± 0.05**	**6.84**	**1, 32.27**	**0.013**

[a] Food intake: two-level fixed factor (low and high food intake).

[b] Full model: specific growth rate = liver COX activity + muscle COX activity + initial body mass + food intake + liver ATP/O ratio + muscle ATP/O ratio + liver Ks + muscle Ks + food intake × liver COX activity + food intake × muscle COX activity + food intake × initial body mass + food intake × liver ATP/O ratio + food intake × muscle ATP/O ratio + food intake × liver Ks + food intake × muscle Ks.

[c] Full model: growth efficiency = liver COX activity + muscle COX activity + initial body mass + food intake + liver ATP/O ratio + muscle ATP/O ratio + liver Ks + muscle Ks + food intake × liver COX activity + food intake × muscle COX activity + food intake × initial body mass + food intake × liver ATP/O ratio + food intake × muscle ATP/O ratio + food intake × liver Ks + food intake × muscle Ks.

[d] Full model: specific protein gain = liver COX activity + muscle COX activity + initial protein mass + food intake + liver ATP/O ratio + muscle ATP/O ratio + liver Ks + muscle Ks + food intake × liver COX activity + food intake × muscle COX activity + food intake × initial protein mass + food intake × liver ATP/O ratio + food intake × muscle ATP/O ratio + food intake × liver Ks + food intake × muscle Ks.

individuals, mitochondrial efficiency is a flexible trait that can change in response to environmental conditions [38,39] and stage of life [34,40]. A higher mitochondrial efficiency may also have a cost because mitochondria are a major producer of reactive oxygen species (ROS) and mitochondrial efficiency can be positively related to ROS production [17,37]. When the generation of ROS in an organism exceeds the capacity of its antioxidant defence and repair mechanisms to combat its effects, there can be an accumulation of oxidative damage [41]. ROS have been proposed as an important factor underlying cellular and whole-organism senescence [41] and therefore, a potential cost linked to fast growth [42,43]. Despite this cost, in some contexts, natural selection may favour phenotypes with relatively high mitochondrial efficiency (because this can lead to faster growth, increased body size at maturity, minimized mortality risk and higher number of eggs), whereas in other contexts, a lower mitochondrial efficiency and decreased ROS production might be beneficial (e.g. under conditions of ad libitum food availability) [7,17,37,44]. This hypothesis is in accordance with several recent studies suggesting that variation in mitochondrial function is a key target of natural selection [45,46].

Our findings that fish with high liver mitochondrial efficiency had a high rate of protein synthesis in their muscles and faster growth match our predictions that a higher efficacy at converting food into ATP can lead to an increased allocation to energetically costly processes such as protein synthesis and growth. Contrary to expectations, the rate of protein synthesis in white muscle was negatively correlated with growth performance; individuals that grew the best displayed lower rates of muscle protein synthesis for a given liver mitochondrial efficiency. An explanation for this discrepancy might lie in the fact that rates of protein synthesis are tissue-specific [47] and the correlation of protein synthesis rates across different tissues in the same individual can be poor (as shown by this study), and so the range of tissues that have been measured in our study might not be representative of the overall rate of protein synthesis in the entire animal, because this would be defined as the sum of the individual tissue-specific rates of protein synthesis [48]. However, positive relationships between protein synthesis in white muscle and body growth have been reported in other species [26,47]. An alternative explanation is based on the fact that body proteins are continually being broken down as well as synthesized, and so protein synthesis will only result in growth if the rate of synthesis exceeds the rate of degradation; it has previously been shown that growth variation among-individual fish is more explained by variation in rates of protein degradation than rates of protein synthesis [26]. While measurements of protein degradation rates were beyond the scope of the present study, it may only be possible to explain observed patterns of protein growth if all aspects of protein metabolism (synthesis and degradation) are considered [49].

In conclusion, our study has demonstrated a clear positive relationship between the efficiency with which liver mitochondria convert energy substrates into ATP and whole-animal growth performance. Future research should focus on quantifying the presumed costs of highly efficient mitochondria. Information on the causes and consequences of variation in mitochondrial efficiency would allow prediction of the consequences for whole-animal performance of variation in mitochondrial function, so linking cellular processes to organismal fitness.

Ethics. All procedures were carried out under the jurisdiction of a UK Home Office project licence (PPL 60/4292).

Data accessibility. The dataset supporting this article are available from the Dryad Digital Repository at: https://doi.org/10.5061/dryad.5c5372c [50].

Authors' contributions. K.S., S.G.L., I.M.C., C.S. and N.B.M. conceived the ideas and designed methodology. K.S., E.M.V., G.J.A., S.G.L. and C.A.M. collected the data. K.S., E.M.V., I.M.C., S.G.L. and C.A.M. analysed the data. K.S. led the writing of the manuscript; S.G.L., I.M.C., C.S. and N.B.M. revised the manuscript and added comments. All authors gave final approval for publication.

Competing interests. The authors declare they have no competing interests.

Funding. This research was supported by a European Research Council Advanced (grant no. 322784) to N.B.M.

Acknowledgements. We thank Graham Law, Ross Phillips and Alastair Kirk for help with fish husbandry.

References

1. Arendt JD. 1997 Adaptive intrinsic growth rates: an integration across taxa. *Q. Rev. Biol.* **72**, 149–177. (doi:10.1086/419764)
2. Stamps JA. 2007 Growth-mortality tradeoffs and 'personality traits' in animals. *Ecol. Lett.* **10**, 355–363. (doi:10.1111/j.1461-0248.2007.01034.x)
3. Dmitriew CM. 2011 The evolution of growth trajectories: what limits growth rate? *Biol. Rev.* **86**, 97–116. (doi:10.1111/j.1469-185X.2010.00136.x)
4. Armstrong DP, Keevil MG, Rollinson N, Brooks RJ. 2018 Subtle individual variation in indeterminate growth leads to major variation in survival and lifetime reproductive output in a long-lived reptile. *Funct. Ecol.* **32**, 752–761. (doi:10.1111/1365-2435.13014)
5. Gregory TR, Wood CM. 1998 Individual variation and interrelationships between swimming performance, growth rate, and feeding in juvenile rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.* **55**, 1583–1590. (doi:10.1139/cjfas-55-7-1583)
6. Auer SK, Salin K, Rudolf AM, Anderson GJ, Metcalfe NB. 2015 The optimal combination of standard metabolic rate and aerobic scope for somatic growth depends on food availability. *Funct. Ecol.* **29**, 479–486. (doi:10.1111/1365-2435.12396)
7. Halsey LG. 2018 Keeping slim when food is abundant: what energy mechanisms could be at play? *Trends Ecol. Evol.* **33**, 745–753. (doi:10.1016/j.tree.2018.08.004)
8. Bottje WG, Carstens GE. 2009 Association of mitochondrial function and feed efficiency in poultry and livestock species. *J. Anim. Sci.* **87**, E48–E63. (doi:10.2527/jas.2008-1379)
9. Salin K, Luquet E, Rey B, Roussel D, Voituron Y. 2012 Alteration of mitochondrial efficiency affects oxidative balance, development and growth in frog (*Rana temporaria*) tadpoles. *J. Exp. Biol.* **215**, 863–869. (doi:10.1242/jeb.062745)
10. Lehninger AL, Nelson DL, Cosx MM. 1993 *Principles of biochemistry*. New York, NY: Worth Publishers.
11. Brand MD. 2005 The efficiency and plasticity of mitochondrial energy transduction. *Biochem. Soc. Trans.* **33**, 897–904. (doi:10.1042/BST0330897)
12. Brand MD, Chien LF, Ainscow EK, Rolfe DFS, Porter RK. 1994 The causes and functions of mitochondrial proton leak. *Biochim. Biophys. Acta Bioenerg.* **1187**, 132–139. (doi:10.1016/0005-2728(94)90099-X)
13. Rolfe DFS, Brand MD. 1996 Contribution of mitochondrial proton leak to skeletal muscle respiration and to standard metabolic rate. *Am. J. Physiol. Cell Physiol.* **271**, C1380–C1389. (doi:10.1152/ajpcell.1996.271.4.C1380)
14. Speakman JR *et al.* 2004 Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. *Aging Cell* **3**, 87–95. (doi:10.1111/j.1474-9728.2004.00097.x)
15. Salin K, Auer SK, Rudolf AM, Anderson GJ, Selman C, Metcalfe NB. 2016 Variation in metabolic rate among individuals is related to tissue-specific differences in mitochondrial leak respiration. *Physiol. Biochem. Zool.* **89**, 511–523. (doi:10.1086/688769)
16. Salin K, Auer SK, Anderson GJ, Selman C, Metcalfe NB. 2016 Inadequate food intake at high temperatures is related to depressed mitochondrial respiratory capacity. *J. Exp. Biol.* **219**(Pt 9), 1356–1362. (doi:10.1242/jeb.133025)
17. Salin K, Auer SK, Rey B, Selman C, Metcalfe NB. 2015 Variation in the link between oxygen consumption and ATP production, and its relevance for animal performance. *Proc. R. Soc. B* **282**, 20151028. (doi:10.1098/rspb.2015.1028)
18. Fraser KPP, Rogers AD. 2007 Protein metabolism in marine animals: the underlying mechanism of growth. In *Advances in marine biology* (ed. C Sheppard), pp. 267–362. Warwick, UK/Amsterdam, The Netherlands: University of Warwick/Elsevier Ltd.
19. Eya JC, Ashame MF, Pomeroy CF, Manning BB, Peterson BC. 2012 Genetic variation in feed consumption, growth, nutrient utilization efficiency and mitochondrial function within a farmed population of channel catfish (*Ictalurus punctatus*). *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **163**, 211–220. (doi:10.1016/j.cbpb.2012.05.019)
20. Iqbal M, Pumford NR, Tang ZX, Lassiter K, Ojano-Dirain C, Wing T, Cooper M, Bottje W. 2005 Compromised liver mitochondrial function and complex activity in low feed efficient broilers are associated with higher oxidative stress and differential protein expression. *Poult. Sci.* **84**, 933–941. (doi:10.1093/ps/84.6.933)
21. Martinez E, Menze MA, Agosta SJ. 2017 Reduced mitochondrial efficiency explains mismatched growth and metabolic rate at supraoptimal temperatures. *Physiol. Biochem. Zool.* **90**, 294–298. (doi:10.1086/689871)
22. Salin K, Roussel D, Rey B, Voituron Y. 2012 David and Goliath: a mitochondrial coupling problem?

J. Exp. Zool. A Ecol. Genet. Physiol. **317**, 283–293. (doi:10.1002/jez.1722)

23. Toyomizu M, Okamoto K, Tanaka M, Ishibashi T. 1992 Effect of 2,4-dinitrophenol on growth and body composition of broilers. *Poult. Sci.* **71**, 1096–1100. (doi:10.3382/ps.0711096)
24. Carlson SM, Olsen EM, Vollestad LA. 2008 Seasonal mortality and the effect of body size: a review and an empirical test using individual data on brown trout. *Funct. Ecol.* **22**, 663–673. (doi:10.1111/j.1365-2435.2008.01416.x)
25. Jonsson N, Jonsson B. 1999 Trade-off between egg mass and egg number in brown trout. *J. Fish Biol.* **55**, 767–783. (doi:10.1111/j.1095-8649.1999.tb00716.x)
26. McCarthy ID, Houlihan DF, Carter CG. 1994 Individual variation in protein turnover and growth efficiency in rainbow trout, *Oncorhynchus mykiss (Walbaum)*. *Proc. R. Soc. Lond. B* **257**, 141–147. (doi:10.1098/rspb.1994.0107)
27. Elliott JM. 1976 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.* **45**, 923–948. (doi:10.2307/3590)
28. X'avia CY, Black CM, Lin AJ, Ping P, Lau E. 2015 Mitochondrial protein turnover: methods to measure turnover rates on a large scale. *J. Mol. Cell. Cardiol.* **78**, 54–61. (doi:10.1016/j.yjmcc.2014.10.012)
29. Garlick PJ, McNurlan MA, Preedy VR. 1980 A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of [^{3}H]phenylalanine. *Biochem. J.* **192**, 719–723. (doi:10.1042/bj1920719)
30. Lamarre SG, Saulnier RJ, Blier PU, Driedzic WR. 2015 A rapid and convenient method for measuring the fractional rate of protein synthesis in ectothermic animal tissues using a stable isotope tracer. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **182**, 1–5. (doi:10.1016/j.cbpb.2014.11.006)
31. Salin K, Villasevil EM, Auer SK, Anderson GJ, Selman C, Metcalfe NB, Chinopoulos C. 2016 Simultaneous measurement of mitochondrial respiration and ATP production in tissue homogenates and calculation of effective P/O ratios. *Physiol. Rep.* **4**, e13007. (doi:10.14814/phy2.13007)
32. Larsen S *et al.* 2012 Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. *J. Physiol.* **590**, 3349–3360. (doi:10.1113/jphysiol.2012.230185)
33. Biro PA, Adriaenssens B, Sampson P. 2014 Individual and sex-specific differences in intrinsic growth rate covary with consistent individual differences in behaviour. *J. Anim. Ecol.* **83**, 1186–1195. (doi:10.1111/1365-2656.12210)
34. Distefano G, Standley RA, Zhang XL, Carnero EA, Yi F, Cornnell HH, Coen PM. 2018 Physical activity unveils the relationship between mitochondrial energetics, muscle quality, and physical function in older adults. *J. Caxhexia Sarcopenia Muscle* **9**, 279–294. (doi:10.1002/jcsm.12272)
35. Toyomizu M, Kikusato M, Kawabata Y, Azad MAK, Inui E, Amo T. 2011 Meat-type chickens have a higher efficiency of mitochondrial oxidative phosphorylation than laying-type chickens. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **159**, 75–81. (doi:10.1016/j.cbpa.2011.01.020)
36. Robert KA, Bronikowski AM. 2010 Evolution of senescence in nature: physiological evolution in populations of garter snake with divergent life histories. *Am. Nat.* **175**, E47–159. (doi:10.1086/649595)
37. Brand MD. 2000 Uncoupling to survive? The role of mitochondrial inefficiency in ageing. *Exp. Gerontol.* **35**, 811–820. (doi:10.1016/S0531-5565(00)00135-2)
38. Strobel A, Graeve M, Poertner HO, Mark FC. 2013 Mitochondrial acclimation capacities to ocean warming and acidification are limited in the antarctic notothenioid fish, *Notothenia rossii* and *Lepidonotothen squamifrons*. *PLoS ONE* **8**, e68865. (doi:10.1371/journal.pone.0068865)
39. Blier PU, Lemieux H, Pichaud N. 2013 Holding our breath in our modern world: will mitochondria keep the pace with climate changes? *Can. J. Zool.* **92**, 591–601. (doi:10.1139/cjz-2013-0183)
40. Conley KE, Jubrias SA, Cress ME, Esselman P. 2013 Exercise efficiency is reduced by mitochondrial uncoupling in the elderly. *Exp. Physiol.* **98**, 768–777. (doi:10.1113/expphysiol.2012.067314)
41. Halliwell B, Gutteridge JMC. 1989 *Free radicals in biology and medicine*. Oxford, UK: Clarendon Press.
42. De Block M, Stoks R. 2008 Compensatory growth and oxidative stress in a damselfly. *Proc. R. Soc. B* **275**, 781–785. (doi:10.1098/rspb.2007.1515)
43. Metcalfe NB, Monaghan P. 2001 Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* **16**, 254–260. (doi:10.1016/S0169-5347(01)02124-3)
44. Salin K, Villasevil EM, Anderson GJ, Auer SK, Selman C, Hartley RC, Mullen W, Chinopoulos C, Metcalfe NB. 2018 Decreased mitochondrial metabolic requirements in fasting animals carry an oxidative cost. *Funct. Ecol.* **32**, 2149–2157. (doi:10.1111/1365-2435.13125)
45. Baris TZ, Wagner DN, Dayan DI, Du X, Blier PU, Pichaud N, Oleksiak MF, Crawford DL. 2017 Evolved genetic and phenotypic differences due to mitochondrial-nuclear interactions. *PLoS Genet.* **13**, e1006517. (doi:10.1371/journal.pgen.1006517)
46. Hood WR *et al.* 2018 The mitochondrial contribution to animal performance, adaptation, and life-history variation. *Integr. Comp. Biol.* **58**, 480–485. (doi:10.1093/icb/icy089)
47. Houlihan DF, McMillan DN, Laurent P. 1986 Growth rates, protein synthesis, and protein degradation rates in rainbow trout: effects of body size. *Physiol. Zool.* **59**, 482–493. (doi:10.2307/30158601)
48. Carter CG, Houlihan DF. 2001. Protein synthesis. In *Fish physiology* (eds PA Wright, PM Anderson), pp. 31–75. San Diego, CA: Academic Press.
49. Morgan IJ, McCarthy ID, Metcalfe NB. 2000 Life-history strategies and protein metabolism in overwintering juvenile Atlantic salmon: growth is enhanced in early migrants through lower protein turnover. *J. Fish Biol.* **56**, 637–647. (doi:10.1111/j.1095-8649.2000.tb00761.x)
50. Salin K, Villasevil EM, Anderson GJ, Lamarre SG, Melanson CA, McCarthy I, Selman C, Metcalfe NB. 2019 Data from: Differences in mitochondrial efficiency explain individual variation in growth performance. Dryad Digital Repository. (https://doi.org/10.5061/dryad.5c5372c)