Genetic coupling of life-history and aerobic performance in Atlantic salmon indicates potential constrains on life-history evolution

Short title: Genetic coupling of life-history and metabolism

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Author contributions

JMP, ERÅ, SM, CRP and TA conceived the ideas and designed methodology; JMP, ERÅ, SM, PB, JE, AR and TA collected the data; JMP, SM and TA analysed the data; JMP and TA led the writing of the manuscript. All authors contributed to the drafts and gave final approval for publication.

Abstract

A better understanding of the genetic and phenotypic architecture underlying life-history variation is a longstanding aim in biology. Theories suggest energy metabolism determines life-history variation by modulating resource acquisition and allocation trade-offs, but the genetic underpinnings of the relationship and its dependence on ecological conditions have rarely been demonstrated. The strong genetic determination of age-at-maturity by two unlinked genomic regions (*vgll3* and *six6*) makes Atlantic salmon (*Salmo salar*) an ideal model to address these questions. Using more than 250 juveniles in common garden conditions, we quantified the covariation between metabolic phenotypes –standard and maximum metabolic rates (SMR and MMR), and aerobic scope (AS) – and the life-history genomic regions and tested if food availability modulates the relationships. We found that the metabolic phenotype was significantly affected by *vgll3*, whereby the early maturation genotype was associated with higher MMR and AS. Additionally, MMR exhibited physiological epistasis; it was decreased when late maturation genotypes co-occurred in both genomic regions. Contrary to the expectation, the life-history genotypes had no effects on SMR, and food availability had no effect on the genetic covariation, suggesting a lack of genotype-by-environment interactions. Our results provide insights on the key organismal processes that link energy use to life-history variation, and the potential metabolic constrains on life-history evolution.

Introduction

Physiological processes control how life-history diversity emerges from resource allocation and acquisition trade-offs (Ricklefs & Wikelski 2002). The rate of aerobic energy metabolism is a pivotal mechanism contributing to life-history variation – it modulates resource acquisition, provides cells with ATP, and constrains energy allocation to different body components and functions. Theories, such as the metabolic theory of ecology and the pace-of-life syndrome theory (Brown *et al.* 2004; Dammhahn *et al.* 2018), suggest metabolic rate covaries with life-history variation within and among species. This covariation may have a genetic basis, consequently constraining trait evolution (Roff 1997), yet only a few studies have demonstrated intraspecific genetic covariation or co-evolution between metabolic rate and life-history traits (Boratynski *et al.* 2013; Krams *et al.* 2017; Auer *et al.* 2018b) (see also Careau *et al.* (2011) and Maiti *et al.* (2019) for behaviour and metabolism). Determining whether this relation is modified by different ecological contexts (e.g., food availability) is crucial to better understand the mechanisms shaping life history variation and demographic shifts in populations in response to environmental changes (Hughes *et al*. 2005; Lailvaux & Husak 2014).

The quintessential components of energy metabolism at the organismal level (i.e., the metabolic phenotypes) are standard metabolic rate (SMR), maximum metabolic rate (MMR), and the absolute aerobic scope (AS) that is the difference between SMR and MMR (Fry 1947; Chabot *et al.* 2016; Metcalfe *et al.* 2016). Together, these components of metabolism modulate resource allocation and acquisition by defining minimal energy requirements and maximal aerobic performance. SMR is the minimum metabolic rate of an ectothermic animal associated with self-maintenance, and therefore defines the minimal cost of living (excluding growth, digestion and locomotion). MMR defines the upper limit of aerobic performance and of AS, which reflects the energy budget that can be allocated into non-maintenance functions, such as locomotion and digestion (Arnott *et al.* 2006; Farrell 2009). Higher MMR and by extension, AS, are predicted to increase fitness via facilitating energetically demanding behaviours (such as migration, aggression, predator avoidance, and prey capture) and tolerance to environmental stress (Clark *et al.* 2011; Eliason *et al.* 2013; Metcalfe *et al*. 2016; Sandblom *et al.* 2016). However, high aerobic performance comes with costs, including maintaining a larger heart and gill surface area (associated with increased demand for osmoregulation) (Priede 1985; Farrell 2009; Killen *et al.* 2016).

Allocation of energy to growth or improved condition can link metabolic phenotypes to life-history traits (Thorpe 2007). Life-history traits, such as the timing of maturation and migration, are determined by adaptive body-size thresholds (Roff 1994; Salminen 1997; Thorpe *et al.* 1998; Theriault *et al.* 2007), and metabolic phenotypes are often correlated with growth rate, albeit in a context-dependent manner (Burton *et al.* 2011; Metcalfe *et al*. 2016). Under high food availability, a high SMR in combination with high AS can increase growth rate (Auer *et al.* 2015c), as it often correlates with traits that improve resource acquisition, such as dominance and digestive capacity (Niemelä & Dingemanse 2018; Mathot *et al*. 2019; Rosenfeld *et al.* 2020). Under low food availability, the growth benefit of high SMR or AS can be minimized (or even reversed for SMR) due to high self-maintenance costs (Auer *et al.* 2015c; Zeng *et al.* 2017; Auer *et al.* 2020b). In addition, individuals can be forced to seek new habitats or take more risks to acquire resources exerting further fitness costs (Killen *et al.* 2011; Auer *et al.* 2020a). Hence, covariation between life-history and metabolism, whether it is genetically or environmentally driven, could be modulated by resource availability. A resource dependent change in genetic covariation, i.e., genotype-by-environment interaction) could maintain genetic variation in these traits (Gillespie & Turelli 1989; Hughes *et al*. 2005; Gutteling *et al.* 2007; Niitepõld 2010) but this has not been demonstrated.

In anadromous (sea-migrating) salmonids, the number of years the fish spends at sea before the first spawning, i.e., sea age-at-maturity, has a dramatic effect on its size-at-maturity (Fleming & Einum 2011): individuals spending one year at sea typically weigh 1–3 kg compared with 10–20 kg after 3 or more years. Increased size in late maturing individuals also translates to marked gains in reproductive investment in both sexes (Fleming & Einum 2011). Age-at-maturity variation is maintained by fitness trade-offs; earlier maturation, i.e., less time spent at sea, provides a fitness advantage through a higher probability of survival prior to reproduction and a shorter generation time, but that comes at the expense of fecundity and mating success (due to smaller size at reproduction (Mobley *et al.* 2021*;* Hendry *et al.* 1999; Stearns 2000). Early maturation depends on faster growth and fat deposition compared to late maturation in the freshwater (juvenile) and early marine phase (Skilbrei 1989; Salminen 1997; Hutchings & Jones 1998; Tréhin *et al.* 2021). This suggests that early maturation in salmonids may be associated with higher SMR or aerobic scope via resource utilization (Mathot *et al*. 2019; Rosenfeld *et al.* 2020).

Across Atlantic salmon (*Salmo salar* L. 1758) populations, a large proportion of variation in age-at-maturity in both sexes is explained by a single genomic region that encompasses the *vgll3* (*vestigial-like 3)* gene on chromosome 25 (Ayllon *et al.* 2015; Barson *et al.* 2015). In addition, variation in another locus on chromosome 9, *six6*, (after *six homeobox 6 gene* in this region), is a strong predictor of mean age-at-maturity among populations (Barson *et al.* 2015), and associated with early maturation in an aquaculture strain of salmon (Sinclair-Waters *et al.* 2020). *Vgll3* and *six6* are also associated with size-at-maturity, with the alleles conferring late maturation being associated with larger age-specific body size especially after multiple years at sea (Barson *et al.* 2015). In the last few decades, many Atlantic salmon populations have been maturing, on average, at younger ages (Chaput 2012), which is associated with an increase in the frequency of the early maturation allele in *vgll3,* in some cases (Czorlich *et al.* 2018). Recently, Czorlich *et al.* (2021) identified a link between the decrease in salmon age-at-maturity and a change in prey species composition (see also Aykanat *et al.* (2020) for diet composition in relation to *six6*). These observations further highlight that genotype dependent differences in performance traits related to metabolism, such as foraging success or food assimilation (Metcalfe *et al.* 1995; Auer *et al.* 2015a), may be related to contemporary life-history evolution in Atlantic salmon.

The strong effects of the *six6* and *vgll3* genomic regions on life-history variation provide an opportunity for the genetic covariation between age-at-maturity and energy metabolism to be studied prior to maturation, i.e., at the juvenile stage, by genetic prediction. This approach makes controlled, empirical settings more feasible, as salmon require several years to reach maturation. In this study, we use genetic prediction of the age at maturity of Atlantic salmon and test if (i) genetic covariation exists between life-history and metabolic phenotypes in juveniles, and (ii) resource limitation can induce genotype-by-environment interactions in metabolic phenotypes. Specifically, we test the hypothesis that resource availability and age-at-maturity genotypes interact in their effects on metabolism; under high food availability, salmon with early maturation genotypes are predicted to show a higher SMR and AS than fish with late maturation genotypes, but under low food availability, the effects of genotypes are predicted to be weaker.

Material and Methods

The experiments were conducted under an animal experiment permit granted by the Finnish Project Authorisation Board (permit nr. ESAVI/4511/2020).

Fish crossing, rearing and genotyping

The parental individuals of Atlantic salmon used in this study were obtained from a first-generation hatchery brood stock (the first generation bred in captivity) originating from the river Kymijoki in Finland, managed by the Natural Resources Institute Finland (LUKE), Laukaa hatchery. In October 2019, families were crossed from eggs and milt collected in the Laukaa hatchery and transferred to the University of Helsinki for fertilisation (parental fish details in Table S1). Full-sib families were created by crossing individuals with heterozygous *vgll3* and *six6* genotypes, i.e., *vgll3**E/L and *six6**E/L, where E and L refer to the alleles associated with early and late maturation, respectively. This provided offspring with all genotype combinations within each full-sib family. Fertilized eggs were incubated in vertical incubators in replicated, family-specific compartments at 7ºC in the dark. Astroturf material was used in the bottom of the incubators.

On 6 March 2020, several weeks before the fry commenced independent feeding, the hatched alevins were transferred to Lammi Biological Station (61°04′45″N, 025°00′40″E, Lammi, Finland, transfer time approx. 2h), where they were released into circular tanks, diameter 90cm. Tank water volume was initially 189L and increased to 306L in July. Each family was reared in a separate tank, supplied with a continuous flow of UV-filtered water from the nearby lake Pääjärvi, creating a slow current in each tank. The water was warmed by 1°C with a heat-exchange system before being pumped into the tanks. Photoperiod was set to match the natural light cycle at the same latitude.

Feeding of alevins was started when most of the egg yolk had been consumed in March 2020. Feed rations were calculated assuming feed conversion efficiency of 0.8, using growth predictions from Elliott & Hurley (1997). Fish were initially fed with 0.2mm commercial feed (Vita, Veronesi), 4 times d^{-1}, and transferred to 0.5mm feed and more frequent feeding, 16 times d^{-1}, gradually until the required feed weight exceeded 8g d^{-1}, after which Profi-Automatic feeders (LINN Gerätebau GmbH, Lennestad, Germany), were deployed. Feeding was continued ‘ad libitum’, i.e., maximizing the number of feeds to up to 16 times d^{-1}

188 (allowing food to be available in the water column for a maximum amount of time while
189 minimizing the amount of leftover feed). Tanks were cleaned by scrubbing surfaces and
190 siphoning excess food once week^{-1} until June 1st, then approximately twice week^{-1} until
191 August 14th, then once week^{-1} until the end of the experiment. Mortality from first feeding in
192 March until PIT-tagging in July was approximately 8%. Water temperature during this time
193 increased from *ca.* 4.5 °C to 11°C (Fig. S1).
194
195 Between 14 and 21 July 2020, fish were captured by netting, anaesthetised with sodium
196 bicarbonate-buffered methanesulfonate (100 mg/L) and individually tagged with passive
197 integrated transponder (PIT) tags (length 8mm, width 1.2mm, Manruta, Quangdong, China)
198 inserted into abdominal cavity with a syringe and needle. Only fish that were >45mm were
199 tagged (Bangs *et al.* 2013). Afterwards, the fish were weighed, photographed (for a separate
200 study), and a small fin clip was collected from their caudal fin using a scalpel. Fish were
201 allowed to recover in aerated buckets briefly after anaesthesia, after which they were returned
202 to the rearing tanks. Total mortality due to tagging/anaesthesia was approx. 5%. After
203 tagging, environmental enrichment was provided to the tanks in the form of stones (diameter
204 approx. 8cm) placed in square, stainless-steel baskets (mesh size 2cm, width and length
205 20cm). Three baskets with three stones in each were provided to each tank.
206
207 The fin clip of each individual was placed in 20 µL of Lucigen QuickExtract DNA Extraction
208 Solution 1.0 and kept on ice or at -20°C until extractions were completed on the day of
209 sample collection according to manufacturer's instructions. DNA was stored at -20°C. DNA
210 was diluted 10-fold in water, after which genotyping was performed using a Kompetitive
211 Allele-Specific PCR (KASP, LGC genomics, UK) assay (He *et al.* 2014) for *vgll3* (Sinclair-
212 Waters *et al.* 2020) and *six6*, as well as for *sdY* to determine sex (details in online
213 supplemental material).
214
215 **Experimental design**
216 At minimum two weeks after PIT-tagging, fish from each family were divided into two tanks
217 at roughly even densities (mean 1.29 g L^{-1}, N = 145–152 tank^{-1}). The experiment (see Fig. 1,
218 created with BioRender.com) started in August 2020, at least three weeks after PIT-tagging
219 for each family. In the beginning of August, the relative age of the fish was approximately
220 2050 degree days, or 1313 Tau as calculated with the formula of Gorodilov (1996).

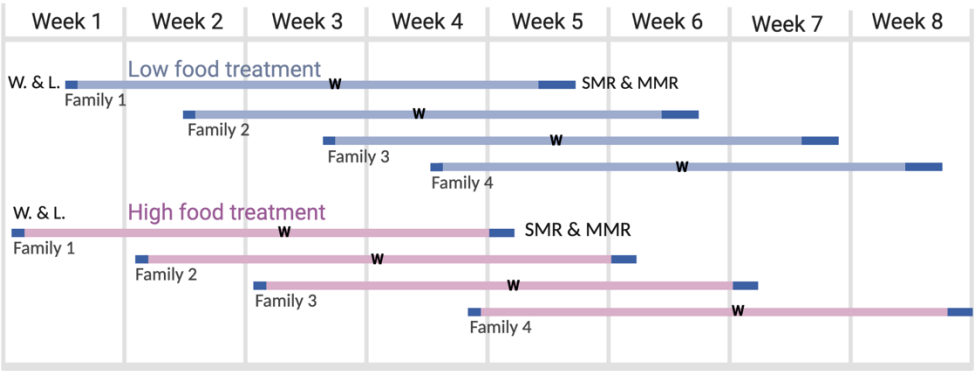


Fig. 1. Timeline of the experiment, starting 03 August 2020. The duration of low food and high food treatments was four weeks for each family. The "W" indicates when fish density of high food tanks was reduced to the level of low food tanks based on mean biomass. Each horizontal line represents a separate tank. Dark blue represents timing of procedures for each tank (W. & L. = weight and length, and/or SMR & MMR measurements).

Low food and high food treatments

One tank from each family was assigned to a feed restriction (hereafter low food) treatment. Immediately before the treatment, these fish had been measured once for SMR (as described below, for a separate study), with in total 3 days fasting, weighed to nearest 0.01g, and measured to nearest mm. During the low food treatment, fish were fed twice week^{-1} using automatic feeders, which distributed the estimated whole daily ration of food to the tank within 2h in 10 doses. Intermittent feeding to satiation was preferred over constant low ration to minimize the formation of strong dominance hierarchies in tanks (Ward *et al*. 2006), and because individual feeding as in, e.g., Auer *et al.* (2015b) was not feasible. Parallel to the low-food treatment, the other tank for each family was assigned to a high food treatment. These fish were weighed and measured before the treatment begun, including 2d fasting and anaesthesia. The high food treatment consisted of the total estimated daily ration, delivered daily in eight doses distributed equally during an 8h period (9:00-17:00) using the automatic feeders. Further details provided in online supplemental material. 28 days after the feeding treatments commenced (range 28–31d because measurements took 2–3 days for each tank), 48 fish from each family (192 in total) in the low food treatment, and 32 fish from the same four families in the high food treatment (128 in total), were measured for their SMR and MMR (Fig. 1), though only 290 homozygous individuals were used in the data analysis

246 (Table S2). Densities of fish biomass in the tanks at the end of the treatments were on average
247 1.8 and 2.8 g L-1 in the low food and high food treatments, respectively.
248
249 *SMR and MMR measurements*
250 Two days before their SMR measurement started, fish for each batch were caught by netting
251 and their genotype was identified using PIT-tags, after which they were moved into an
252 acclimation tank (Fig. S3). Each batch contained 16 individuals from the same family and
253 tank, and was balanced for all homozygous genotype-sex-combinations (in most cases N = 2
254 group^{-1} batch^{-1}, with fish heterozygous for either locus used when homozygous fish were not
255 available). Fish of desired genotypes were randomly picked from the rearing tanks by netting,
256 apart from the high food treatment from the 4th family where some fish had grown too large
257 for the respirometers. All fish from this tank were anaesthetised, weighed, and measured four
258 days before their respirometry trials, and appropriate size fish (max. length 82mm) were
259 selected for the trials from each genotype (*vgll3* and *six6* genotype frequencies were not
260 different between these two size groups, Chi^2= 5.5, df = 7, p = 0.6).
261
262 During acclimation, the fish were held individually, without feeding, in 20×20×10cm cages
263 made from stainless steel frame, black phthalate-free mosquito net, and hot glue, with a net
264 cover. The aim of the acclimation period was to minimize the effects of potential daily
265 temperature fluctuations in rearing tanks (which obtained water from the nearby lake, hence
266 daily temperature fluctuations occurred, Fig. S1), digestion, growth and social interactions on
267 SMR (Rosenfeld *et al.* 2015). The temperature of acclimation tanks was set to 11°C ± 0.1°C
268 (details in online supplemental material).
269
270 We measured SMR using intermittent flow respirometry (Forstner 1983; Svendsen *et al.*
271 2016; Killen *et al.* 2021). The SMR measurements were started after the fish had spent 42–
272 47h in acclimation, between 11:30 and 14:30. SMR was measured until 8:00 the following
273 day, after which we measured the MMR of the fish as post-exercise oxygen consumption
274 using a chase method, similarly to, e.g., Raby *et al.* (2020), where MMR reflects increased
275 aerobic respiration related to exercise and the oxygen debt incurred by anaerobic respiration
276 (Brett 1964; Norin & Clark 2016). Although this method may underestimate MMR compared
277 to swimming respirometry (Raby *et al.* 2020), it was the preferred method for small juvenile
278 salmon, which do not actively swim against a current in a swimming respirometer (personal

observation). After the MMR measurements, fish were euthanized with an overdose of methanesulfonate and then measured, weighed and dissected. All fish were in an immature state based on appearance of gonads. After each family was measured (5d of measurements), the respirometer was cleaned with 10% bleach and all chamber parts scrubbed to limit bacterial growth, followed by thorough rinsing with water. For details on the SMR and MMR measurements, see online supplementary material.

Growth rate was calculated as specific (a.k.a. instantaneous) growth rate as follows: [(ln mass at t_2 - ln body mass at t_1) / days between t_1 and t_2)] × 100, where t is date. Fulton's condition factor was calculated as (body mass / length^3) × 100.

Analysis of respirometry data

Based on blank tests conducted before and after the SMR measurement, background respiration was accounted for using the R package *FishResp* (Morozov *et al.* 2019), assuming linear growth of microorganisms inside a respirometry chamber over time (only weak background respiration was observed). Oxygen consumption rate (MO_2, mg O_2 h^{-1}) for each linear measurement phase, represented as a slope, was derived from best-fit linear regression of dissolved oxygen concentration over time (details in online supplementary material). The mean of the lowest normal distribution (MLND) was used to estimate SMR from the extracted MO_2 slopes (Chabot *et al.* 2016).

MMR was calculated from the O_2 concentrations (mg O_2 L^{-1}) after background correction was performed in *FishResp* (Morozov *et al.* 2019). We used two methods to identify the slope of the steepest decrease in O_2 saturation. First, we used package *respR* (Harianto *et al.* 2019) with the function *auto_rate*, fitting one and two-minute windows (Little *et al.* 2020) (example slope in Fig. S5A). Second, slopes for MMR were extracted using a derivative of a polynomial curve fitted on each measurement (function *smooth.spline,* df=10). This is the '*spline-MMR*' method (Fig. S5B). The slopes were then used to calculate MMR in mg O_2 h^{-1} using *FishResp*-package function *calculate.MR*. MMR values calculated by the 1-min *respR* and *spline-MMR* approaches were highly correlated (Pearson-r = 0.98, 95% confidence interval 0.98 – 0.99). We selected the spline-MMR data for further analysis. Absolute aerobic scope (AS, the difference of MMR and SMR) was calculated using the MLND SMR data and

311 the spline-MMR data. For further details on SMR and MMR analyses, see online
312 supplementary information and data availability.
313
314 **Statistical analyses**
315 To test for the effects of treatment and genotype on metabolic variables, we ran separate
316 linear mixed models using SMR, MMR, and AS as response variables. This mixed model
317 framework allowed us to account for variation arising from i) fish body size, ii) family effects
318 (background genetic variation between families and tank effects), iii) timing of experiments
319 in relation to photoperiod and temperature during rearing, i.e., batch effects, iv) and technical
320 variation related to MMR measurements. Only individuals homozygous for both *vgll3* and
321 *six6* were included in the analysis because individuals heterozygous for either locus were not
322 measured from all families.
323
324 The response variables and body mass as a covariate were $\log_{10}$-transformed to account for
325 allometric scaling of metabolic rate. We included treatment, *vgll3* and *six6* genotypes, and
326 sex as fixed effects in all models. Random effects for SMR included family and measurement
327 batch. For MMR and AS they included family and the chamber where MMR was measured.
328 The chamber effect accounted for random variation among persons performing the chase
329 (half of the trials were consistently performed by the same person, J.P., the rest were divided
330 among three people), and variation due to the timing of the chase test (chamber order was
331 always the same when placing fish into chambers with only a few exceptions). Inclusion of
332 the name of person performing the chase as a random effect did not change the results of the
333 analysis or decrease the residual variance. Batch was not included as a random effect in
334 MMR and AS models due to model singularity. To test if genotype-specific metabolic rates
335 were affected by sex –because females mature later than males in both early and late age-at-
336 maturity genotypes– and by food availability, i.e., to test for genotype-by-environment
337 interaction, we fitted additional interactions into the models, including pairwise interactions
338 between *vgll3* and *six6* genotypes, between genotypes and treatment, and between genotypes
339 and sex. Further, the interaction of $\log_{10}$ body mass with treatment was included to test for
340 potential treatment-specific allometric scaling of metabolic rate, which may be related to
341 body composition differences between treatments. The full models were reduced by omitting
342 non-significant interactions in a stepwise process based on Type III test p-values to obtain
343 estimates for significant interaction effects. When interactions between fixed genotype effects
344 were significant, p-values for the pairwise differences between genotypes were obtained by

post hoc analysis using package *emmeans* (Lenth 2020). A summary of model parameters is shown in Table S3.

All models were fitted with package *lme4* v. 1.1-26 (Bates *et al.* 2015) in R v. 3.6.2 (R Core Team 2019) with an alpha-value 0.05. P- and F-test values for fixed effects were computed using type III tests with Satterthwaite's method. Residuals of models were confirmed to be homoscedastic and normally distributed; only the residuals of SMR between the high food and low food treatments were slightly heteroscedastic, which may have made the test more conservative, but the residuals between sexes and genotypes were homoscedastic. Outliers were identified with function *outlierTest* (Bonferroni-corrected $p < 0.05$, package *car*). One outlier was identified and removed from each of SMR and MMR, after which models were rerun. Predicted means were obtained with function *ggpredict* in package *ggeffects* (Lüdecke 2018). The data were visualized using *ggplot2* v.3.3.3 (Wickham 2016) and *interactions* (Long 2019). Pearson's correlation coefficient among mass- and family-corrected SMR, MMR and AS were calculated using residuals from a mixed model with each response variable $\log_{10}$ transformed, with $\log_{10}$-transformed body mass as covariate and with family as random effect.

Results

Low food treatment decreased both the specific growth rate and condition factor of the fish compared to high food treatment (Fig. S6). The mean body length of fish was 70.6 ± 4.5 and 66.2 ± 4.9mm (SD), and the mean body mass was 4.2 ± 0.8 and 3.3 ± 0.8g after the high and low food treatment, respectively.

Standard metabolic rate

There was no significant genotype, food availability or sex effect on SMR (Table 1, Fig. 2). There was a marginally significant interaction effect of *six6* and food availability on SMR ($p = 0.045$ in the full model, $p = 0.055$ in the simplified model, Table S4), but none of the pairwise contrasts were significant (the largest effect being: *six6* EE-genotype, high food vs. low food, $t_{25.6} = -2.37$, $p = 0.11$). The metabolic scaling exponent, *b*, i.e., the slope of log SMR with log body mass, was significantly higher in the high food treatment (0.94, $R^2 = 0.73$) than in the low food treatment (0.87, $R^2 = 0.69$) (Fig. S7a, $p = 0.025$, Table 1).

377 **Maximum metabolic rate**

378 Fish with the *vgll3* early maturation genotype had a higher MMR than fish with the late
379 maturation genotype (Fig. 2, Table 1). *Vgll3* genotype also interacted with *six6*, such that
380 MMR was decreased when late maturation genotypes of the two loci cooccurred compared to
381 other genotype combinations (Fig. 2, Table 1). None of the treatment-genotype or sex-
382 genotype interactions or the main effects of sex or food availability had a significant effect on
383 MMR (Table S5). Unlike in SMR, the metabolic scaling of MMR was not significantly
384 affected by food treatment (b = 0.86, R^2 = 0.76).

385

386 **Aerobic scope**

387 Fish with the *vgll3* early maturation genotype had an approximately 4.5% higher AS
388 compared to fish with the late-maturation genotype (Fig. 2, Table 1, predicted means 458.9
389 and 439.2 mg O_2 kg^{-1} h^{-1} for early and late maturation genotypes, respectively). AS was
390 increased by the low food availability compared to high food availability, but only in smaller
391 fish (interaction p = 0.049, Table 1); scaling exponent b = 0.94 (R^2 = 0.57) in the high food
392 and 0.90 (R^2 = 0.68) in the low food treatment (Fig. S7b). The *vgll3* and food treatment
393 effects were also significant when mass adjusted SMR was included as a covariate in the
394 model (Table S6), indicating that the genotype effect was independent of SMR. The *six6* or
395 sex effects were not significant, and there were no significant interaction effects between
396 genotypes and treatment or sex on AS (full model in Table S7).

397

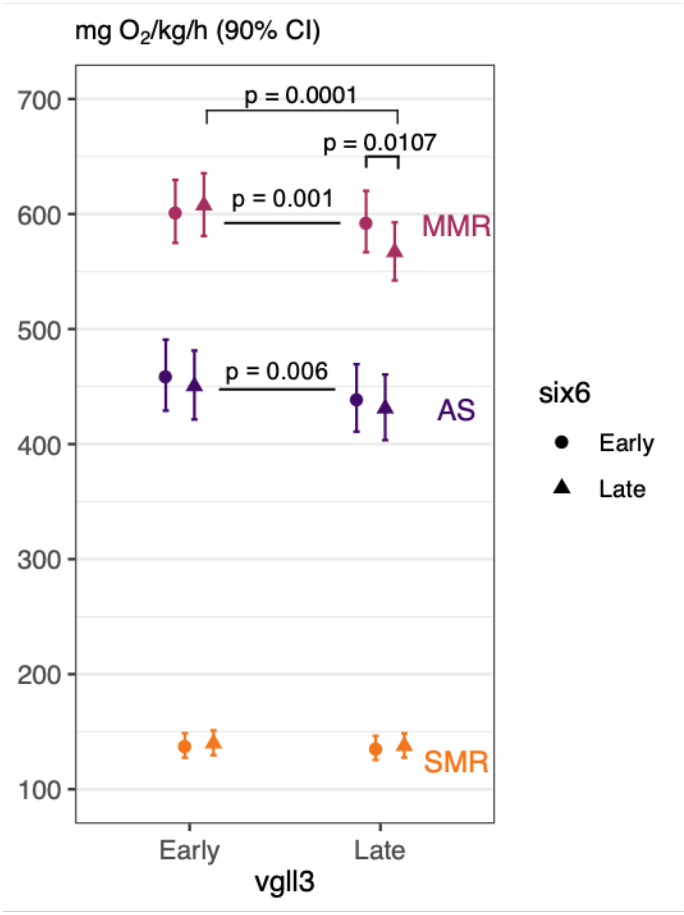


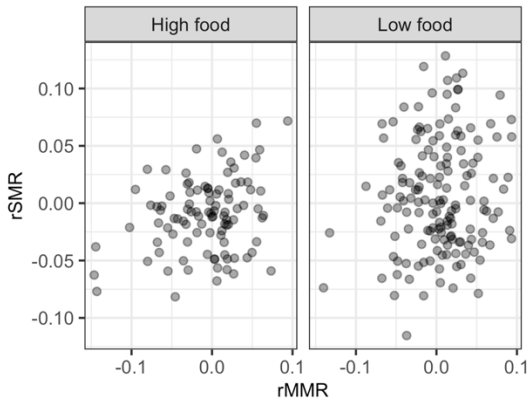
Fig 2. Predicted means for standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS) in *vgll3* and *six6* early- and late-maturation genotypes with 90% confidence intervals. The means are predicted for a female, 3.7g individual in the high food treatment. Values were back transformed to linear scale. P-values show significant pairwise differences between genotypes for MMR on top of the points, and for the *vgll3* main effects in MMR and AS between the points. N = 60–71 in each genotype combination (same individuals used for all traits).

Table 1. Simplified linear mixed models for log_{10}-transformed metabolic phenotypes. Full models are shown in the supplemental material. P -values obtained by Type III test (Satterthwaite's method).

Response variable	Coefficient	Estimate	SE	SSq	Den DF	F (type III)	p
SMR	Intercept	-0.797	0.036				
	Treatment (low food)	-0.055	0.036	0.003	241.85	2.34	0.127
	Sex (male)	0.002	0.005	0.0002	248.04	0.14	0.706
	Vgll3 (LL)	-0.007	0.005	0.003	252.11	1.82	0.179
	Six6 (LL)	-0.005	0.005	0.001	248.00	0.98	0.324
	Log_{10} body mass	**0.897**	**0.049**	**1.498**	**258.76**	**1026.08**	**<0.0001**
	Treatment × log_{10} body mass	**0.135**	**0.06**	**0.007**	**258.83**	**5.09**	**0.025**
	Random effect	**Var**	**2.5% C.I.**	**97.5% C.I.**			
	Batch	0.0005	0.0002	0.0012			
	Family	0.0013	0.0003	0.0081			
	Residual	0.0015					
MMR	Intercept	-0.133	0.021				
	Treatment (low food)	0.007	0.006	0.002	248.56	1.27	0.262
	Sex (male)	-0.004	0.005	0.001	253.69	0.57	0.451
	***Vgll3* (LL)**	**-0.006**	**0.008**	**0.021**	**252.73**	**12.05**	**0.001**
	Six6 (LL)	0.004	0.007	0.004	253.82	2.05	0.154
	Log_{10} body mass	**0.847**	**0.029**	**1.429**	**255.78**	**827.34**	**<0.0001**
	Vgll3 (LL) × Six6 (LL)	**-0.024**	**0.010**	**0.009**	**252.37**	**5.11**	**0.025**
	Random effect	**Var**	**2.5% C.I.**	**97.5% C.I.**			
	Chamber	0.0003	0.0001	0.0007			
	Family	0.0003	0.0001	0.0020			
	Residual	0.0017					
AS	Intercept	-0.308	0.044				
	Treatment (low food)	**0.104**	**0.048**	**0.013**	**245.78**	**4.64**	**0.032**
	Sex (male)	-0.006	0.007	0.002	241.96	0.68	0.412
	***Vgll3* (LL)**	**-0.019**	**0.007**	**0.022**	**241.36**	**7.79**	**0.006**
	Six6 (LL)	-0.008	0.007	0.004	243.55	1.38	0.241
	Log_{10} body mass	**0.947**	**0.066**	**1.266**	**244.34**	**452.65**	**<0.0001**
	Treatment × log_{10} body mass	**-0.163**	**0.082**	**0.011**	**245.94**	**3.92**	**0.049**
	Random effect	**Var**	**2.5% C.I.**	**97.5% C.I.**			
	Chamber	0.0004	0.0001	0.0012			
	Family	0.0008	0.0002	0.0053			
	Residual	0.0028					

Correlations among metabolic phenotypes

409 There was a positive correlation between mass- and family- corrected SMR and MMR in the
410 high food, but not the low food treatment (Fig. 3), and a very strong correlation between
411 MMR and AS in both treatments (Table 2).



412

413 Fig 3. Scatter plots of family- and mass-corrected SMR and MMR in high and low food
414 treatments. Dots represent individuals. Correlation coefficients are shown in Table 2. N = 100
415 (high food) and 153 (low food).

416

Table 2. Pearson's correlation coefficients between metabolic phenotypes in high food (above diagonal) and low food (below diagonal) treatments. P-values given in parentheses.

	rSMR	rMMR	rAbsAS
rSMR		**0.28 (0.004)**	0.13 (0.09)
rMMR	0.11 (0.16)		**0.99 (<0.001)**
rAbsAS	**-0.17 (0.04)**	**0.96 (<0.001)**	

417

418 # Discussion

419 High growth and accumulation of energy reserves in the juvenile stage leads to faster
420 maturation in salmonids (Skilbrei 1989; Hutchings & Jones 1998) and in several other taxa
421 (Frisch 1985; Weimerskirch 1992; Cheung *et al.* 1997). Here, we found that the *vgll3* early
422 maturation genotype increased the aerobic scope (AS) of juvenile Atlantic salmon compared
423 to the late maturation genotype. A previous study showed that higher condition factor,
424 mediated by the *vgll3* early maturation genotype, positively affected the initiation of male
425 parr maturation (Debes *et al.* 2021). The results presented here suggest superior resource
426 acquisition or assimilation via higher AS as a potential mechanism by which an increased
427 condition factor in *vgll3* early maturation -genotype males could be achieved compared to

428 late maturation -genotype conspecifics. The two-locus system system in our study was found
429 to exhibit physiological epistasis (Cheverud & Routman 1995), as the cooccurrence of the
430 late maturing genotypes in both loci resulted in lower maximum metabolic rate (MMR) than
431 their additive effects. The epistasis may help to maintain genetic variation under rapid
432 adaptive responses (Cheverud & Routman 1996; Merilä & Sheldon 1999). The functional
433 effects of the different genotypes that may explain the epistasis are not well known, but both
434 *six6* and *vgll3* are expressed during development and have been implicated in the control of
435 cell fate commitment and the hypothalamus-pituitary-gonad axis in salmon (Kurko *et al.*
436 2020; Moustakas-Verho *et al.* 2020). Furthermore, although genetic variation in both
437 genomic regions is a strong predictor of the age structure of populations at maturity, the effect
438 of *six6* is diminished after correcting for population stratification (Barson *et al.* 2015),
439 suggesting a functional difference in their effects on age-at-maturity. Hence, the epistatic
440 interaction between two genomic regions may stem from, e.g., developmental canalisation
441 (Debat & David 2001) that leads to functional alterations in morphology and physiology in
442 different genotype combinations. The *vgll3* genomic region is the major genetic axis
443 explaining variation in age-at-maturity in salmon, and *vgll3* variation is also spatially
444 divergent among populations and under rapid adaptative evolution (Barson *et al.* 2015;
445 Czorlich *et al.* 2018; Pritchard *et al.* 2018; Zueva *et al.* 2021). Thus, addressing the causal
446 physiological and morphological mechanisms of the link between the genomic regions and
447 aerobic performance will shed light into the mechanisms of life history evolution in salmon.
448
449 Unlike AS and MMR, SMR did not exhibit *vgll3*-linked covariation with age-at-maturity.
450 Likewise, a decoupling of SMR and AS in relation to life-history variation was found by
451 Archer *et al.* (2020) in resident and migratory brown trout (*Salmo trutta*), and MMR (a strong
452 predictor of AS in our study), but not SMR, was positively selected for in Atlantic salmon
453 under high food competition (Auer *et al.* 2018a). A lack of differences in SMR across the
454 *vgll3* genotypes was also found in our parallel study, in which the fish were smaller (mean
455 ~1g) compared to this study (mean ~4g) (Åsheim *et al.* 2021). Nevertheless, the result is
456 unexpected, as SMR, or basal metabolic rate in endotherms, has been proposed to explain
457 life-history variation along the fast-slow axis (Boratynski *et al.* 2013; Auer *et al.* 2018b), but
458 see (Polverino *et al.* 2018). Even though we do not rule out the possibility of SMR affecting
459 variation in age-at-maturity phenotypically or via small-effect loci (Johnston *et al.* 2014;
460 Sinclair-Waters *et al.* 2020), our results suggest that the genetic control of maturation by the
461 *vgll3* genomic region via AS mostly involves physiological pathways that do not alter SMR

simultaneously, at least at this developmental stage. Such pathways may be related to oxygen demand by tissues or its supply (uptake, transport, or unloading) during stress and/or exhaustive exercise. For example, structural and functional variation in the heart (i.e., cardiac output) or muscle (Gamperl & Farrell 2004; Anttila & Mänttäri 2009), and mechanisms that modulate oxygen carrying capacity of the cardiovascular system might invoke changes in AS without altering SMR (Harter *et al.* 2019; Nikinmaa *et al.* 2019; McArley *et al.* 2021). The observed low and lacking correlation between SMR and MMR in the high and low food treatments, respectively, also emphasises that SMR and MMR are mostly related to different underlying traits.

The timing of maturation, just as many life-history traits, depends on reaching a certain body size threshold, i.e., the acquisition of sufficient energy that can be allocated for maturation processes (Moghadam *et al.* 2007; Taranger *et al.* 2010; Verta *et al.* 2020). Because of context-dependent covariation between metabolism and growth rate (see meta-analysis by Auer *et al.* (2020b)), we tested if resource availability modified the genetic covariation between metabolic phenotypes and age-at-maturity. Against our predictions, our experiments did not reveal a change in SMR or MMR, or genotype-by-environment interactions, due to feed restriction, despite a strong decrease in growth rate. The average increase in AS observed in the low food compared to high food availability was specific to small size classes, and possibly related to lower relative amount of adipose tissue that may affect the body mass -adjusted AS. The lack of genotype-by-environment interactions indicates that salmon age-at-maturity genotypes coped equally well with the resource variation we applied. The lack of SMR response likely indicates that the metabolic activity of tissues did not respond to the feeding regime we had implemented (Norin & Malte 2012). However, a stronger feed deprivation could have resulted in a more pronounced effect on SMR, as found in other studies (O'Connor *et al.* 2000; Auer *et al.* 2015b). Our low food treatment included approximately 3 days of fasting in between feeding to satiation, similar to a “feast and famine” feeding strategy (Armstrong & Schindler 2011). A lack of metabolic response to reduced food availability may be beneficial if it allows the individual to maximize acquisition via food assimilation when this strategy is used.

Salmon in the wild are increasingly experiencing higher than optimal temperatures due to climate change (Friedland *et al.* 2009), and MMR is typically less plastic than SMR in response to environmental temperature (Sandblom *et al.* 2016). Thus, the differences in

496 aerobic scope between *vgll3* early and late maturation genotypes may be reflected in the
497 resource acquisition and growth of juvenile salmon at elevated temperatures. Under these
498 conditions, higher aerobic scope may enable higher feeding capacity (Auer *et al.* 2015a),
499 because a temperature-related reduction in aerobic scope due to increased SMR and specific
500 dynamic action (the post-feeding increase in metabolic rate) could reduce appetite in fishes,
501 as is hypothesized in Jutfelt *et al.* (2021). Thus, our results indicate a potentially important
502 advantage for individuals carrying the early maturation genotype under global warming. This
503 advantage may also extend to higher survival of early maturing salmon relative to late
504 maturing salmon in their spawning migration if the genotype effect on AS persists across life-
505 stages (Clark *et al.* 2011; Eliason *et al.* 2011; Mottola *et al.* 2020). This may link aerobic
506 performance to demographic changes in salmon populations. It can potentially also be
507 relevant to survival of salmon after spawning, and thereby repeated spawning (iteroparity),
508 because iteroparity tendency is co-inherited with the same *vgll3* genotype as early maturation
509 (Aykanat *et al.* 2019).
510
511 Salmon males mature on average earlier than females (Fleming & Einum 2011) and the *vgll3*
512 locus is associated with freshwater maturation in male parr (Debes *et al.* 2021; Verta *et al.*
513 2020). Other studies in salmonids have found sex differences in the performance of 2+ year-
514 old or mature individuals, including a higher aerobic scope, but not SMR, and a higher cost
515 of swimming in males than in females (Clark *et al.* 2011; Makiguchi *et al.* 2017; Archer *et al.*
516 2020). The lack of sex differences in metabolic phenotypes in our study both across and
517 within age-at-maturity genotypes, therefore, supports the conclusion that sex-dependent life-
518 history variation is not reflected in metabolic rates during the juvenile stage, as suggested by
519 a few other studies (Regnier *et al.* 2015; Prokkola *et al.* 2021; Åsheim *et al.* 2021). Recently,
520 it was also shown that salmon females have a higher size threshold for maturation after one
521 year at sea compared to males (Tréhin *et al.* 2021), and further studies are required to link the
522 performance differences and sex-specific maturation schedules of salmon at sea.
523
524 Our experiment focussed on the genetic component determining age-at-maturity – rearing
525 fish until maturation was out of our scope, excluding the possibility to evaluate the
526 environmental component. The presence of genetic covariation between aerobic scope and
527 age-at-maturity constrains evolution because selection acting on either trait would alter the
528 phenotypic variation of the other (Lande 1979; Roff 1997). For example, natural selection
529 favouring later age-at-maturity would indirectly constrain the aerobic scope of juveniles to a

530 lower level, even if that may be a suboptimal phenotype. On the other hand, the genetic
531 covariation between aerobic scope at the juvenile stage and age-at-maturity may help
532 maintain optimal trait variation in age-at-maturity, e.g., by constraining the potentially
533 maladaptive environmentally induced variation in age-at-maturity (e.g., De Jong 1999; Tufto
534 2000). River geophysical properties are important determinants of the optimal age structure
535 of populations at maturity, whereby populations in smaller tributaries have a younger and
536 populations in large, fast-flowing rivers have an older age structure (Fleming & Einum 2011).
537 Therefore, forecasting the optimal age-at-maturity from juvenile phenotypic performance
538 (i.e., growth) would be maladaptive for salmon individuals if covariation between
539 performance traits and age at maturity was explained entirely by environmental effects. It is
540 possible that genetic covariation between juvenile performance and life-history variation also
541 occurs in other taxa in which environmental characteristics are associated with optimal life-
542 history strategies.
543
544 Understanding the physiological basis of life-history variation in different life-stages and
545 environmental conditions can provide insights into the factors driving life-history evolution,
546 and hence, better predictions of the responses of populations to environmental changes. Wild
547 salmon populations have declined in recent decades, with a concomitant decrease in the
548 frequency of late maturing individuals in Arctic populations (Friedland *et al.* 2009; Czorlich
549 *et al.* 2018). Our study provides evidence that salmon that have a genetic tendency for late
550 maturation also have a genetic tendency for reduced aerobic scope in the juvenile stage
551 compared to salmon with a genetic tendency for early maturation, indicating that evolution
552 towards an earlier age-at-maturity can cause correlated selection towards increased aerobic
553 scope. In conclusion, our study demonstrates an eco-physiological approach to functionally
554 validate adaptive genes identified from genomic scans and emphasizes the value of better
555 integration of aerobic scope into the theoretical predictions on life-history variation.
556
557 **Data availability**
558 The data from metabolic rate measurements are available in Zenodo
559 (10.5281/zenodo.5153281). The R codes for SMR, MMR and statistical analysis are available
560 in an archived repository (v.1.0.0, doi: 10.5281/zenodo.5168998).
561
562 **Conflicts of interest**
563 The authors declare no conflicts of interest.

Supplemental Material

Supplemental material: Pdf-file with Material and Methods, Figures, and Tables.

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