Genetic coupling of life-history and aerobic performance in juvenile Atlantic salmon

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

1. The physiological underpinnings of life history adaptations in ectotherms are not well understood. Theories suggest energy metabolism influences life history variation via modulation of resource acquisition. However, the genetic basis of this relation and its dependence on ecological conditions, such as food availability, have rarely been characterized, despite being critical to predicting the responses of populations to environmental changes.
2. The Atlantic salmon (*Salmo salar*) is an emerging wild model species for addressing these questions; strong genetic determination of age-at-maturity at two unlinked genomic regions (*vgll3* and *six6*) enables the use of complex experimental designs and tests of hypotheses on the physiological and genetic basis of life-history trait variation.
3. In this study, we crossed salmon to obtain individuals with all combinations of late and early maturation genotypes for *vgll3* and *six6* within full-sib families. Using more than 250 juveniles in common garden conditions, we tested (i) whether metabolic phenotypes (i.e., standard and maximum metabolic rates, and absolute aerobic scope) were correlated with the age-at-maturity genotypes and (ii) if high vs. low food availability modulated the relationship.
4. We found that salmon with *vgll3* early maturation genotype had a higher aerobic scope and maximum metabolic rate, but not standard metabolic rate, compared to salmon with *vgll3* late maturation genotype. This suggests that physiological or structural pathways regulating maximum oxygen supply or demand are potentially important for the determination of age-at-maturity in Atlantic salmon.
5. *Vgll3* and *six6* exhibited physiological epistasis, whereby maximum metabolic rate significantly decreased when late maturation genotypes were present concurrently in both loci compared to other genotype combinations.

6. The growth of the feed restricted group decreased substantially compared to the high food group. However, the effects of life-history genomic regions on metabolic phenotypes were similar in both feeding regimes, indicating a lack of genotype-by-environment interactions.
7. Our results indicate that aerobic performance of juvenile salmon may affect their age-at-maturity. The results may help to better understand the mechanistic basis of life-history variation, and the 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 7°C 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}

(allowing food to be available in the water column for a maximum amount of time while minimizing the amount of leftover feed). Tanks were cleaned by scrubbing surfaces and siphoning excess food once week^{-1} until June 1st, then approximately twice week^{-1} until August 14th, then once week^{-1} until the end of the experiment. Mortality from first feeding in March until PIT-tagging in July was approximately 8%. Water temperature during this time increased from *ca.* 4.5 °C to 11°C (Fig. S1).

Between 14 and 21 July 2020, fish were captured by netting, anaesthetised with sodium bicarbonate-buffered methanesulfonate (100 mg/L) and individually tagged with passive integrated transponder (PIT) tags (length 8mm, width 1.2mm, Manruta, Quangdong, China) inserted into abdominal cavity with a syringe and needle. Only fish that were >45mm were tagged (Bangs *et al.* 2013). Afterwards, the fish were weighed, photographed (for a separate study), and a small fin clip was collected from their caudal fin using a scalpel. Fish were allowed to recover in aerated buckets briefly after anaesthesia, after which they were returned to the rearing tanks. Total mortality due to tagging/anaesthesia was approx. 5%. After tagging, environmental enrichment was provided to the tanks in the form of stones (diameter approx. 8cm) placed in square, stainless-steel baskets (mesh size 2cm, width and length 20cm). Three baskets with three stones in each were provided to each tank.

The fin clip of each individual was placed in 20 µL of Lucigen QuickExtract DNA Extraction Solution 1.0 and kept on ice or at -20°C until extractions were completed on the day of sample collection according to manufacturer's instructions. DNA was stored at -20°C. DNA was diluted 10-fold in water, after which genotyping was performed using a Kompetitive Allele-Specific PCR (KASP, LGC genomics, UK) assay (He *et al.* 2014) for *vgll3* (Sinclair-Waters *et al.* 2020) and *six6*, as well as for *sdY* to determine sex (details in online supplemental material).

Experimental design

At minimum two weeks after PIT-tagging, fish from each family were divided into two tanks at roughly even densities (mean 1.29 g L^{-1}, N = 145–152 tank^{-1}). The experiment (see Fig. 1, created with BioRender.com) started in August 2020, at least three weeks after PIT-tagging for each family. In the beginning of August, the relative age of the fish was approximately 2050 degree days, or 1313 Tau as calculated with the formula of Gorodilov (1996).

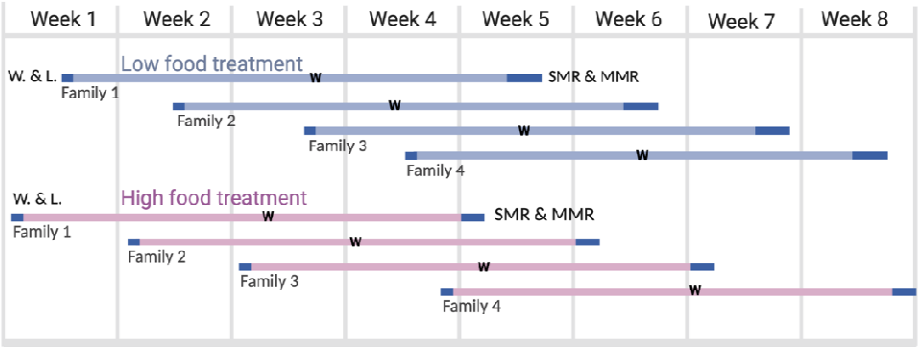

Fig. 1. Timeline of the experiment, starting 03 August 2020. The duration of low food and high food treatments was four weeks for each family. The "W" indicates when fish density of high food tanks was reduced to the level of low food tanks based on mean biomass. Each horizontal line represents a separate 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

(Table S2). Densities of fish biomass in the tanks at the end of the treatments were on average 1.8 and 2.8 g L-1 in the low food and high food treatments, respectively.

SMR and MMR measurements

Two days before their SMR measurement started, fish for each batch were caught by netting and their genotype was identified using PIT-tags, after which they were moved into an acclimation tank (Fig. S3). Each batch contained 16 individuals from the same family and tank, and was balanced for all homozygous genotype-sex-combinations (in most cases N = 2 group^{-1} batch^{-1}, with fish heterozygous for either locus used when homozygous fish were not available). Fish of desired genotypes were randomly picked from the rearing tanks by netting, apart from the high food treatment from the 4th family where some fish had grown too large for the respirometers. All fish from this tank were anaesthetised, weighed, and measured four days before their respirometry trials, and appropriate size fish (max. length 82mm) were selected for the trials from each genotype (*vgll3* and *six6* genotype frequencies were not different between these two size groups, Chi^2= 5.5, df = 7, p = 0.6).

During acclimation, the fish were held individually, without feeding, in 20×20×10cm cages made from stainless steel frame, black phthalate-free mosquito net, and hot glue, with a net cover. The aim of the acclimation period was to minimize the effects of potential daily temperature fluctuations in rearing tanks (which obtained water from the nearby lake, hence daily temperature fluctuations occurred, Fig. S1), digestion, growth and social interactions on SMR (Rosenfeld *et al.* 2015). The temperature of acclimation tanks was set to 11°C ± 0.1°C (details in online supplemental material).

We measured SMR using intermittent flow respirometry (Forstner 1983; Svendsen *et al.* 2016; Killen *et al.* 2021). The SMR measurements were started after the fish had spent 42–47h in acclimation, between 11:30 and 14:30. SMR was measured until 8:00 the following day, after which we measured the MMR of the fish as post-exercise oxygen consumption using a chase method, similarly to, e.g., Raby *et al.* (2020), where MMR reflects increased aerobic respiration related to exercise and the oxygen debt incurred by anaerobic respiration (Brett 1964; Norin & Clark 2016). Although this method may underestimate MMR compared to swimming respirometry (Raby *et al.* 2020), it was the preferred method for small juvenile 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)] $\times$ 100, where t is date. Fulton's condition factor was calculated as (body mass / length^3) $\times$ 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

the spline-MMR data. For further details on SMR and MMR analyses, see online supplementary information and data availability.

Statistical analyses

To test for the effects of treatment and genotype on metabolic variables, we ran separate linear mixed models using SMR, MMR, and AS as response variables. This mixed model framework allowed us to account for variation arising from i) fish body size, ii) family effects (background genetic variation between families and tank effects), iii) timing of experiments in relation to photoperiod and temperature during rearing, i.e., batch effects, iv) and technical variation related to MMR measurements. Only individuals homozygous for both *vgll3* and *six6* were included in the analysis because individuals heterozygous for either locus were not measured from all families.

The response variables and body mass as a covariate were $\log_{10}$-transformed to account for allometric scaling of metabolic rate. We included treatment, *vgll3* and *six6* genotypes, and sex as fixed effects in all models. Random effects for SMR included family and measurement batch. For MMR and AS they included family and the chamber where MMR was measured. The chamber effect accounted for random variation among persons performing the chase (half of the trials were consistently performed by the same person, J.P., the rest were divided among three people), and variation due to the timing of the chase test (chamber order was always the same when placing fish into chambers with only a few exceptions). Inclusion of the name of person performing the chase as a random effect did not change the results of the analysis or decrease the residual variance. Batch was not included as a random effect in MMR and AS models due to model singularity. To test if genotype-specific metabolic rates were affected by sex –because females mature later than males in both early and late age-at-maturity genotypes– and by food availability, i.e., to test for genotype-by-environment interaction, we fitted additional interactions into the models, including pairwise interactions between *vgll3* and *six6* genotypes, between genotypes and treatment, and between genotypes and sex. Further, the interaction of $\log_{10}$ body mass with treatment was included to test for potential treatment-specific allometric scaling of metabolic rate, which may be related to body composition differences between treatments. The full models were reduced by omitting non-significant interactions in a stepwise process based on Type III test p-values to obtain estimates for significant interaction effects. When interactions between fixed genotype effects 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).

Maximum metabolic rate

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

Aerobic scope

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

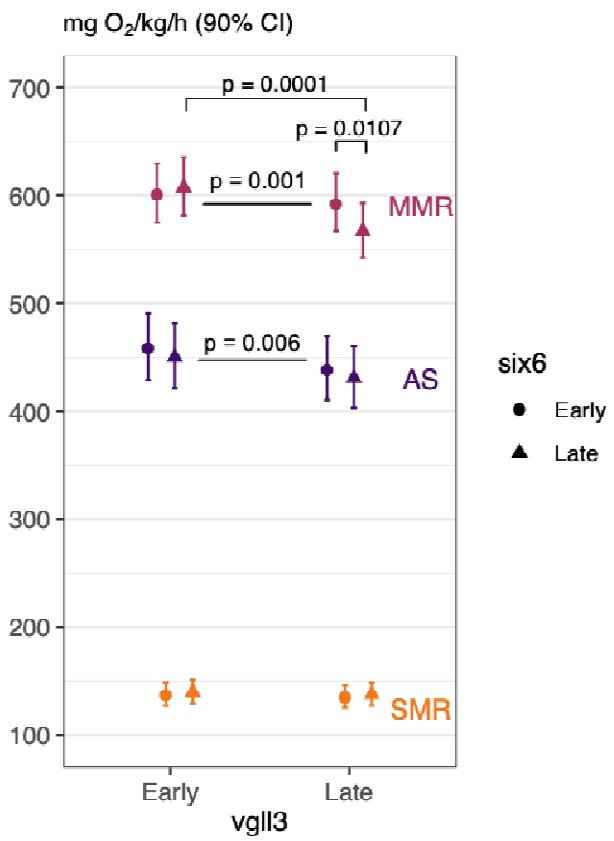

Fig 2. Predicted means for standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS) in *vgll3* and *six6* early- and late-maturation genotypes with 90% confidence intervals. The means are predicted for a female, 3.7g individual in the high food treatment. Values were back transformed 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

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

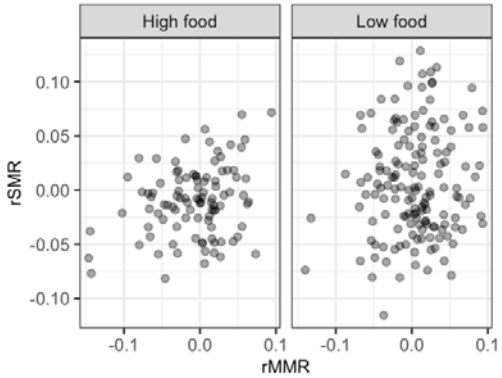

Fig 3. Scatter plots of family- and mass-corrected SMR and MMR in high and low food treatments. Dots represent individuals. Correlation coefficients are shown in Table 2. N = 100 (high food) and 153 (low food).

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)**	

Discussion

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

our study, we also demonstrated physiological epistasis (Cheverud & Routman 1995) between the *vgll3* and *six6* genomic regions, as the cooccurrence of the late maturing genotypes in both loci resulted in lower maximum metabolic rate (MMR) than their additive effects. The epistasis may help to maintain genetic variation under rapid adaptive responses (Cheverud & Routman 1996; Merilä & Sheldon 1999). The functional effects of the different genotypes that may explain the epistasis are not well known, but both *six6* and *vgll3* are expressed during development and have been implicated in the control of cell fate commitment and the hypothalamus-pituitary-gonad axis in salmon (Kurko *et al.* 2020; Moustakas-Verho *et al.* 2020). Furthermore, although genetic variation in both genomic regions is a strong predictor of the age structure of populations at maturity, the effect of *six6* is diminished after correcting for population stratification (Barson *et al.* 2015), suggesting a functional difference in their effects on age-at-maturity. Hence, the epistatic interaction between two genomic regions may stem from, e.g., developmental canalisation (Debat & David 2001) that leads to functional alterations in morphology and physiology in different genotype combinations. The *vgll3* genomic region is the major genetic axis explaining variation in age-at-maturity in salmon, and *vgll3* variation is also spatially divergent among populations and under rapid adaptative evolution (Barson *et al.* 2015; Czorlich *et al.* 2018; Pritchard *et al.* 2018; Zueva *et al.* 2021). Thus, addressing the causal physiological and morphological mechanisms of the link between the genomic regions and aerobic performance will shed light into the mechanisms of life history evolution in salmon.

Unlike AS and MMR, SMR did not exhibit *vgll3*-linked covariation with age-at-maturity. Likewise, a decoupling of SMR and AS in relation to life-history variation was found by Archer *et al.* (2020) in resident and migratory brown trout (*Salmo trutta*), and MMR (a strong predictor of AS in our study), but not SMR, was positively selected for in Atlantic salmon under high food competition (Auer *et al.* 2018a). A lack of differences in SMR across the *vgll3* genotypes was also found in a parallel study, in which the fish were smaller (mean ~1g) compared to this study (mean ~4g) (Åsheim *et al.* 2021). Nevertheless, the result is unexpected, as SMR, or basal metabolic rate in endotherms, has been proposed to explain life-history variation along the fast-slow axis (Boratynski *et al.* 2013; Auer *et al.* 2018b), but see (Polverino *et al.* 2018). Even though we do not rule out the possibility of SMR affecting variation in age-at-maturity phenotypically or via small-effect loci (Johnston *et al.* 2014; Sinclair-Waters *et al.* 2020), our results suggest that the genetic control of maturation by the *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

aerobic scope between *vgll3* early and late maturation genotypes may be reflected in the resource acquisition and growth of juvenile salmon at elevated temperatures. Under these conditions, higher aerobic scope may enable higher feeding capacity (Auer *et al.* 2015a), because a temperature-related reduction in aerobic scope due to increased SMR and specific dynamic action (the post-feeding increase in metabolic rate) could reduce appetite in fishes, as is hypothesized in Jutfelt *et al.* (2021). Thus, our results indicate a potentially important advantage for individuals carrying the early maturation genotype under global warming. This advantage may also extend to higher survival of early maturing salmon relative to late maturing salmon in their spawning migration if the genotype effect on AS persists across life-stages (Clark *et al.* 2011; Eliason *et al.* 2011; Mottola *et al.* 2020). This may link aerobic performance to demographic changes in salmon populations. It can potentially also be relevant to survival of salmon after spawning, and thereby repeated spawning (iteroparity), because iteroparity tendency is co-inherited with the same *vgll3* genotype as early maturation (Aykanat *et al.* 2019).

Salmon males mature on average earlier than females (Fleming & Einum 2011) and the *vgll3* locus is associated with freshwater maturation in male parr (Debes *et al.* 2021; Verta *et al.* 2020). Other studies in salmonids have found sex differences in the performance of 2+ year-old or mature individuals, including a higher aerobic scope, but not SMR, and a higher cost of swimming in males than in females (Clark *et al.* 2011; Makiguchi *et al.* 2017; Archer *et al.* 2020). The lack of sex differences in metabolic phenotypes in our study both across and within age-at-maturity genotypes, therefore, supports the conclusion that sex-dependent life-history variation is not reflected in metabolic rates during the juvenile stage, as suggested by a few other studies (Regnier *et al.* 2015; Prokkola *et al.* 2021; Åsheim *et al.* 2021). Recently, it was also shown that salmon females have a higher size threshold for maturation after one year at sea compared to males (Tréhin *et al.* 2021), and further studies are required to link the performance differences and sex-specific maturation schedules of salmon at sea.

Our experiment focussed on the genetic component determining age-at-maturity – rearing fish until maturation was out of our scope, excluding the possibility to evaluate the environmental component. The presence of genetic covariation between aerobic scope and age-at-maturity constrains evolution because selection acting on either trait would alter the phenotypic variation of the other (Lande 1979; Roff 1997). For example, natural selection favouring later age-at-maturity would indirectly constrain the aerobic scope of juveniles to a

lower level, even if that may be a suboptimal phenotype. On the other hand, the genetic covariation between aerobic scope at the juvenile stage and age-at-maturity may help maintain optimal trait variation in age-at-maturity, e.g., by constraining the potentially maladaptive environmentally induced variation in age-at-maturity (e.g., De Jong 1999; Tufto 2000). River geophysical properties are important determinants of the optimal age structure of populations at maturity, whereby populations in smaller tributaries have a younger and populations in large, fast-flowing rivers have an older age structure (Fleming & Einum 2011). Therefore, forecasting the optimal age-at-maturity from juvenile phenotypic performance (i.e., growth) would be maladaptive for salmon individuals if covariation between performance traits and age at maturity was explained entirely by environmental effects.

Understanding the physiological basis of life-history variation in different life-stages and environmental conditions can provide insights into the factors driving life-history evolution, and hence, better predictions of the responses of populations to environmental changes. Wild salmon populations have declined in recent decades, with a concomitant decrease in the frequency of late maturing individuals in Arctic populations (Friedland *et al.* 2009; Czorlich *et al.* 2018). Our study provides evidence that salmon that have a genetic tendency for late maturation also have a genetic tendency for reduced aerobic scope in the juvenile stage compared to salmon with a genetic tendency for early maturation, indicating that evolution towards an earlier age-at-maturity can cause correlated selection towards increased aerobic scope. In conclusion, our study emphasizes the value of better integration of aerobic scope into theoretical predictions on life-history variation (Lailvaux & Husak 2014; Rosenfeld *et al.* 2020) and demonstrates Atlantic salmon as a genetically tractable system for addressing the physiological and genetic relationships between traits underlying life-history variation.

Data availability

The data from metabolic rate measurements are available in Zenodo (10.5281/zenodo.5153281). The R codes for SMR, MMR and statistical analysis are available in an archived repository (v.1.0.0, doi: 10.5281/zenodo.5168998).

Conflicts of interest

The authors declare no conflicts of interest.

Supplemental Material

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

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