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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Acknowledgements
We thank Nikolai Piavchenko, Katja Maamela, Markus Lauha, Petra Liljeström, Suvi Ikonen, Mikko Immonen, and Anna Toikkanen for fish care and technical help during the experiment, and Lammi Biological Station for a high-quality research environment. Dr. Markus Haapala and the Nordic University Hub NordicPOP (Nordforsk, project no. 85352) are acknowledged for the 3D printing service, and Heidrikur Bergsson for sharing the chamber cap model. The study was funded by Academy of Finland (T. Aykanat: 325964, 1328860, C. R. Primmer: 284941, 286334, 314254 and 314255), the European Research Council under the European Articles Union's Horizon 2020 research and innovation program (grant no. 742312), and the University of Helsinki.

Keywords: age-at-maturity, energetics, metabolic rate, life history evolution

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 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 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}

203 (allowing food to be available in the water column for a maximum amount of time while
204 minimizing the amount of leftover feed). Tanks were cleaned by scrubbing surfaces and
205 siphoning excess food once week^{-1} until June 1st, then approximately twice week^{-1} until
206 August 14th, then once week^{-1} until the end of the experiment. Mortality from first feeding in
207 March until PIT-tagging in July was approximately 8%. Water temperature during this time
208 increased from *ca.* 4.5 °C to 11°C (Fig. S1).
209
210 Between 14 and 21 July 2020, fish were captured by netting, anaesthetised with sodium
211 bicarbonate-buffered methanesulfonate (100 mg/L) and individually tagged with passive
212 integrated transponder (PIT) tags (length 8mm, width 1.2mm, Manruta, Quangdong, China)
213 inserted into abdominal cavity with a syringe and needle. Only fish that were >45mm were
214 tagged (Bangs *et al.* 2013). Afterwards, the fish were weighed, photographed (for a separate
215 study), and a small fin clip was collected from their caudal fin using a scalpel. Fish were
216 allowed to recover in aerated buckets briefly after anaesthesia, after which they were returned
217 to the rearing tanks. Total mortality due to tagging/anaesthesia was approx. 5%. After
218 tagging, environmental enrichment was provided to the tanks in the form of stones (diameter
219 approx. 8cm) placed in square, stainless-steel baskets (mesh size 2cm, width and length
220 20cm). Three baskets with three stones in each were provided to each tank.
221
222 The fin clip of each individual was placed in 20 µL of Lucigen QuickExtract DNA Extraction
223 Solution 1.0 and kept on ice or at -20°C until extractions were completed on the day of
224 sample collection according to manufacturer's instructions. DNA was stored at -20°C. DNA
225 was diluted 10-fold in water, after which genotyping was performed using a Kompetitive
226 Allele-Specific PCR (KASP, LGC genomics, UK) assay (He *et al.* 2014) for *vgll3* (Sinclair-
227 Waters *et al.* 2020) and *six6*, as well as for *sdY* to determine sex (details in online
228 supplemental material).
229
230 **Experimental design**
231 At minimum two weeks after PIT-tagging, fish from each family were divided into two tanks
232 at roughly even densities (mean 1.29 g L^{-1}, N = 145–152 tank^{-1}). The experiment (see Fig. 1,
233 created with BioRender.com) started in August 2020, at least three weeks after PIT-tagging
234 for each family. In the beginning of August, the relative age of the fish was approximately
235 2050 degree days, or 1313 Tau as calculated with the formula of Gorodilov (1996).

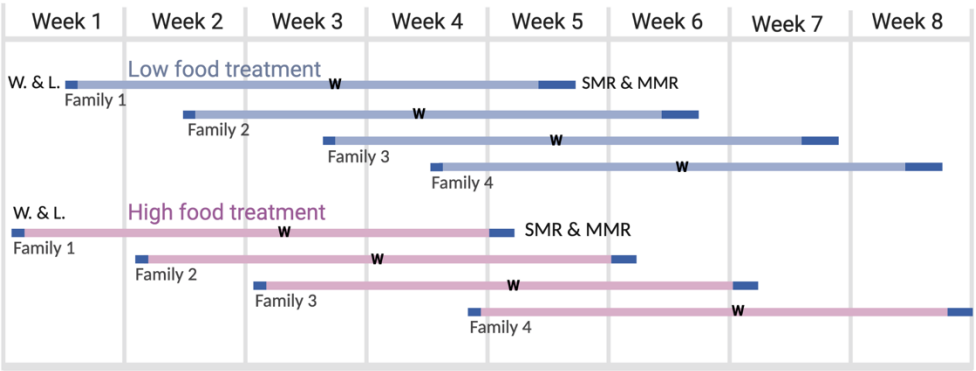

Fig. 1. Timeline of the experiment, starting 03 August 2020. The duration of low food and high food treatments was four weeks for each family. The "W" indicates when fish density of high food tanks was reduced to the level of low food tanks based on mean biomass. Each horizontal line represents a separate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

261 (Table S2). Densities of fish biomass in the tanks at the end of the treatments were on average
262 1.8 and 2.8 g L-1 in the low food and high food treatments, respectively.
263
264 *SMR and MMR measurements*
265 Two days before their SMR measurement started, fish for each batch were caught by netting
266 and their genotype was identified using PIT-tags, after which they were moved into an
267 acclimation tank (Fig. S3). Each batch contained 16 individuals from the same family and
268 tank, and was balanced for all homozygous genotype-sex-combinations (in most cases N = 2
269 group^{-1} batch^{-1}, with fish heterozygous for either locus used when homozygous fish were not
270 available). Fish of desired genotypes were randomly picked from the rearing tanks by netting,
271 apart from the high food treatment from the 4th family where some fish had grown too large
272 for the respirometers. All fish from this tank were anaesthetised, weighed, and measured four
273 days before their respirometry trials, and appropriate size fish (max. length 82mm) were
274 selected for the trials from each genotype (*vgll3* and *six6* genotype frequencies were not
275 different between these two size groups, Chi^2= 5.5, df = 7, p = 0.6).
276
277 During acclimation, the fish were held individually, without feeding, in 20×20×10cm cages
278 made from stainless steel frame, black phthalate-free mosquito net, and hot glue, with a net
279 cover. The aim of the acclimation period was to minimize the effects of potential daily
280 temperature fluctuations in rearing tanks (which obtained water from the nearby lake, hence
281 daily temperature fluctuations occurred, Fig. S1), digestion, growth and social interactions on
282 SMR (Rosenfeld *et al.* 2015). The temperature of acclimation tanks was set to 11°C ± 0.1°C
283 (details in online supplemental material).
284
285 We measured SMR using intermittent flow respirometry (Forstner 1983; Svendsen *et al.*
286 2016; Killen *et al.* 2021). The SMR measurements were started after the fish had spent 42–
287 47h in acclimation, between 11:30 and 14:30. SMR was measured until 8:00 the following
288 day, after which we measured the MMR of the fish as post-exercise oxygen consumption
289 using a chase method, similarly to, e.g., Raby *et al.* (2020), where MMR reflects increased
290 aerobic respiration related to exercise and the oxygen debt incurred by anaerobic respiration
291 (Brett 1964; Norin & Clark 2016). Although this method may underestimate MMR compared
292 to swimming respirometry (Raby *et al.* 2020), it was the preferred method for small juvenile
293 salmon, which do not actively swim against a current in a swimming respirometer (personal

294 observation). After the MMR measurements, fish were euthanized with an overdose of
295 methanesulfonate and then measured, weighed and dissected. All fish were in an immature
296 state based on appearance of gonads. After each family was measured (5d of measurements),
297 the respirometer was cleaned with 10% bleach and all chamber parts scrubbed to limit
298 bacterial growth, followed by thorough rinsing with water. For details on the SMR and MMR
299 measurements, see online supplementary material.
300
301 Growth rate was calculated as specific (a.k.a. instantaneous) growth rate as follows: [(ln mass
302 at t_2 - ln body mass at t_1) / days between t_1 and t_2)] × 100, where t is date. Fulton's condition
303 factor was calculated as (body mass / length^3) × 100.
304
305 **Analysis of respirometry data**
306 Based on blank tests conducted before and after the SMR measurement, background
307 respiration was accounted for using the R package *FishResp* (Morozov *et al.* 2019), assuming
308 linear growth of microorganisms inside a respirometry chamber over time (only weak
309 background respiration was observed). Oxygen consumption rate (MO_2, mg O_2 h^{-1}) for each
310 linear measurement phase, represented as a slope, was derived from best-fit linear regression
311 of dissolved oxygen concentration over time (details in online supplementary material). The
312 mean of the lowest normal distribution (MLND) was used to estimate SMR from the
313 extracted MO_2 slopes (Chabot *et al.* 2016).
314
315 MMR was calculated from the O_2 concentrations (mg O_2 L^{-1}) after background correction
316 was performed in *FishResp* (Morozov *et al.* 2019). We used two methods to identify the
317 slope of the steepest decrease in O_2 saturation. First, we used package *respR* (Harianto *et al.*
318 2019) with the function *auto_rate*, fitting one and two-minute windows (Little *et al.* 2020)
319 (example slope in Fig. S5A). Second, slopes for MMR were extracted using a derivative of a
320 polynomial curve fitted on each measurement (function *smooth.spline,* df=10). This is the
321 '*spline-MMR*' method (Fig. S5B). The slopes were then used to calculate MMR in mg O_2 h^{-1}
322 using *FishResp*-package function *calculate.MR*. MMR values calculated by the 1-min *respR*
323 and *spline-MMR* approaches were highly correlated (Pearson-r = 0.98, 95% confidence
324 interval 0.98 – 0.99). We selected the spline-MMR data for further analysis. Absolute aerobic
325 scope (AS, the difference of MMR and SMR) was calculated using the MLND SMR data and

326 the spline-MMR data. For further details on SMR and MMR analyses, see online
327 supplementary information and data availability.
328
329 **Statistical analyses**
330 To test for the effects of treatment and genotype on metabolic variables, we ran separate
331 linear mixed models using SMR, MMR, and AS as response variables. This mixed model
332 framework allowed us to account for variation arising from i) fish body size, ii) family effects
333 (background genetic variation between families and tank effects), iii) timing of experiments
334 in relation to photoperiod and temperature during rearing, i.e., batch effects, iv) and technical
335 variation related to MMR measurements. Only individuals homozygous for both *vgll3* and
336 *six6* were included in the analysis because individuals heterozygous for either locus were not
337 measured from all families.
338
339 The response variables and body mass as a covariate were $\log_{10}$-transformed to account for
340 allometric scaling of metabolic rate. We included treatment, *vgll3* and *six6* genotypes, and
341 sex as fixed effects in all models. Random effects for SMR included family and measurement
342 batch. For MMR and AS they included family and the chamber where MMR was measured.
343 The chamber effect accounted for random variation among persons performing the chase
344 (half of the trials were consistently performed by the same person, J.P., the rest were divided
345 among three people), and variation due to the timing of the chase test (chamber order was
346 always the same when placing fish into chambers with only a few exceptions). Inclusion of
347 the name of person performing the chase as a random effect did not change the results of the
348 analysis or decrease the residual variance. Batch was not included as a random effect in
349 MMR and AS models due to model singularity. To test if genotype-specific metabolic rates
350 were affected by sex –because females mature later than males in both early and late age-at-
351 maturity genotypes– and by food availability, i.e., to test for genotype-by-environment
352 interaction, we fitted additional interactions into the models, including pairwise interactions
353 between *vgll3* and *six6* genotypes, between genotypes and treatment, and between genotypes
354 and sex. Further, the interaction of $\log_{10}$ body mass with treatment was included to test for
355 potential treatment-specific allometric scaling of metabolic rate, which may be related to
356 body composition differences between treatments. The full models were reduced by omitting
357 non-significant interactions in a stepwise process based on Type III test p-values to obtain
358 estimates for significant interaction effects. When interactions between fixed genotype effects
359 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).

392 **Maximum metabolic rate**

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

400

401 **Aerobic scope**

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

412

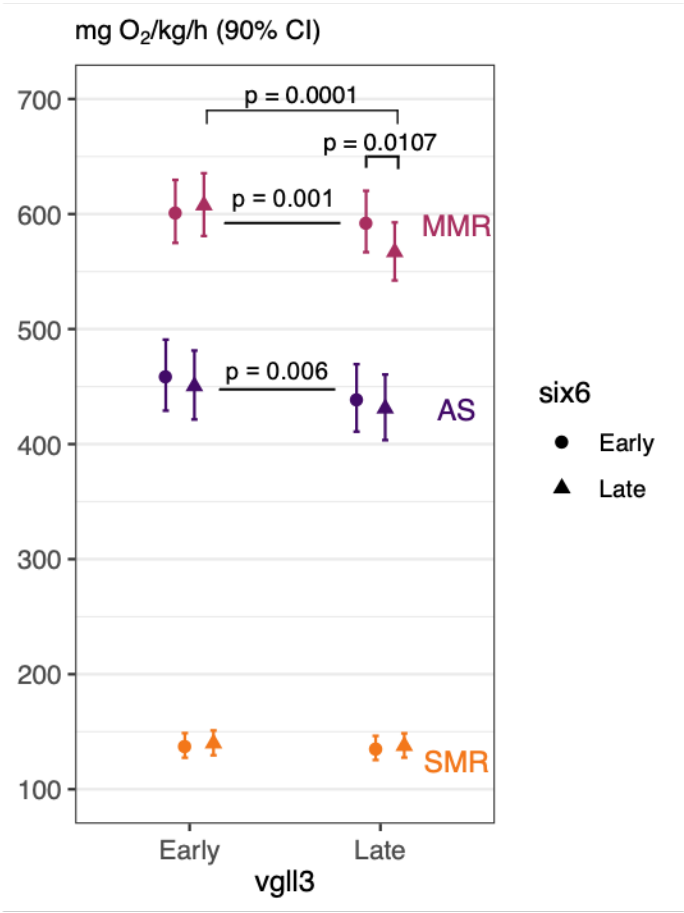

Fig 2. Predicted means for standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS) in *vgll3* and *six6* early- and late-maturation genotypes with 90% confidence intervals. The means are predicted for a female, 3.7g individual in the high food treatment. Values were back transformed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

421

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					

422

423 **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).

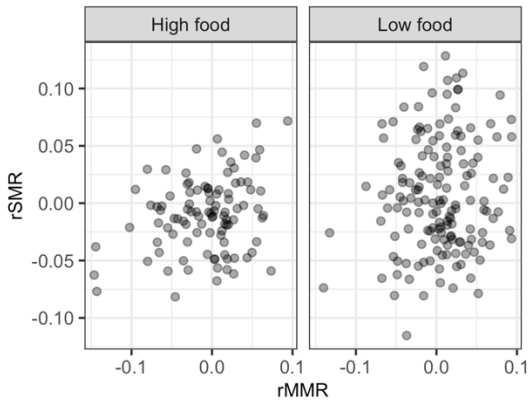

Fig 3. Scatter plots of family- and mass-corrected SMR and MMR in high and low food treatments. Dots represent individuals. Correlation coefficients are shown in Table 2. N = 100 (high food) and 153 (low food).

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

443 our study, we also demonstrated physiological epistasis (Cheverud & Routman 1995)
444 between the *vgll3* and *six6* genomic regions, as the cooccurrence of the late maturing
445 genotypes in both loci resulted in lower maximum metabolic rate (MMR) than their additive
446 effects. The epistasis may help to maintain genetic variation under rapid adaptive responses
447 (Cheverud & Routman 1996; Merilä & Sheldon 1999). The functional effects of the different
448 genotypes that may explain the epistasis are not well known, but both *six6* and *vgll3* are
449 expressed during development and have been implicated in the control of cell fate
450 commitment and the hypothalamus-pituitary-gonad axis in salmon (Kurko *et al.* 2020;
451 Moustakas-Verho *et al.* 2020). Furthermore, although genetic variation in both genomic
452 regions is a strong predictor of the age structure of populations at maturity, the effect of *six6*
453 is diminished after correcting for population stratification (Barson *et al.* 2015), suggesting a
454 functional difference in their effects on age-at-maturity. Hence, the epistatic interaction
455 between two genomic regions may stem from, e.g., developmental canalisation (Debat &
456 David 2001) that leads to functional alterations in morphology and physiology in different
457 genotype combinations. The *vgll3* genomic region is the major genetic axis explaining
458 variation in age-at-maturity in salmon, and *vgll3* variation is also spatially divergent among
459 populations and under rapid adaptative evolution (Barson *et al.* 2015; Czorlich *et al.* 2018;
460 Pritchard *et al.* 2018; Zueva *et al.* 2021). Thus, addressing the causal physiological and
461 morphological mechanisms of the link between the genomic regions and aerobic performance
462 will shed light into the mechanisms of life history evolution in salmon.
463
464 Unlike AS and MMR, SMR did not exhibit *vgll3*-linked covariation with age-at-maturity.
465 Likewise, a decoupling of SMR and AS in relation to life-history variation was found by
466 Archer *et al.* (2020) in resident and migratory brown trout (*Salmo trutta*), and MMR (a strong
467 predictor of AS in our study), but not SMR, was positively selected for in Atlantic salmon
468 under high food competition (Auer *et al.* 2018a). A lack of differences in SMR across the
469 *vgll3* genotypes was also found in a parallel study, in which the fish were smaller (mean ~1g)
470 compared to this study (mean ~4g) (Åsheim *et al.* 2021). Nevertheless, the result is
471 unexpected, as SMR, or basal metabolic rate in endotherms, has been proposed to explain
472 life-history variation along the fast-slow axis (Boratynski *et al.* 2013; Auer *et al.* 2018b), but
473 see (Polverino *et al.* 2018). Even though we do not rule out the possibility of SMR affecting
474 variation in age-at-maturity phenotypically or via small-effect loci (Johnston *et al.* 2014;
475 Sinclair-Waters *et al.* 2020), our results suggest that the genetic control of maturation by the
476 *vgll3* genomic region via AS mostly involves physiological pathways that do not alter SMR

477 simultaneously, at least at this developmental stage. Such pathways may be related to oxygen
478 demand by tissues or its supply (uptake, transport, or unloading) during stress and/or
479 exhaustive exercise. For example, structural and functional variation in the heart (i.e., cardiac
480 output) or muscle (Gamperl & Farrell 2004; Anttila & Mänttäri 2009), and mechanisms that
481 modulate oxygen carrying capacity of the cardiovascular system might invoke changes in AS
482 without altering SMR (Harter *et al.* 2019; Nikinmaa *et al.* 2019; McArley *et al.* 2021). The
483 observed low and lacking correlation between SMR and MMR in the high and low food
484 treatments, respectively, also emphasises that SMR and MMR are mostly related to different
485 underlying traits.
486
487 The timing of maturation, just as many life-history traits, depends on reaching a certain body
488 size threshold, i.e., the acquisition of sufficient energy that can be allocated for maturation
489 processes (Moghadam *et al.* 2007; Taranger *et al.* 2010; Verta *et al.* 2020). Because of
490 context-dependent covariation between metabolism and growth rate (see meta-analysis by
491 Auer *et al.* (2020b)), we tested if resource availability modified the genetic covariation
492 between metabolic phenotypes and age-at-maturity. Against our predictions, our experiments
493 did not reveal a change in SMR or MMR, or genotype-by-environment interactions, due to
494 feed restriction, despite a strong decrease in growth rate. The average increase in AS
495 observed in the low food compared to high food availability was specific to small size
496 classes, and possibly related to lower relative amount of adipose tissue that may affect the
497 body mass -adjusted AS. The lack of genotype-by-environment interactions indicates that
498 salmon age-at-maturity genotypes coped equally well with the resource variation we applied.
499 The lack of SMR response likely indicates that the metabolic activity of tissues did not
500 respond to the feeding regime we had implemented (Norin & Malte 2012). However, a
501 stronger feed deprivation could have resulted in a more pronounced effect on SMR, as found
502 in other studies (O'Connor *et al.* 2000; Auer *et al.* 2015b). Our low food treatment included
503 approximately 3 days of fasting in between feeding to satiation, similar to a “feast and
504 famine” feeding strategy (Armstrong & Schindler 2011). A lack of metabolic response to
505 reduced food availability may be beneficial if it allows the individual to maximize acquisition
506 via food assimilation when this strategy is used.
507
508 Salmon in the wild are increasingly experiencing higher than optimal temperatures due to
509 climate change (Friedland *et al.* 2009), and MMR is typically less plastic than SMR in
510 response to environmental temperature (Sandblom *et al.* 2016). Thus, the differences in

511 aerobic scope between *vgll3* early and late maturation genotypes may be reflected in the
512 resource acquisition and growth of juvenile salmon at elevated temperatures. Under these
513 conditions, higher aerobic scope may enable higher feeding capacity (Auer *et al.* 2015a),
514 because a temperature-related reduction in aerobic scope due to increased SMR and specific
515 dynamic action (the post-feeding increase in metabolic rate) could reduce appetite in fishes,
516 as is hypothesized in Jutfelt *et al.* (2021). Thus, our results indicate a potentially important
517 advantage for individuals carrying the early maturation genotype under global warming. This
518 advantage may also extend to higher survival of early maturing salmon relative to late
519 maturing salmon in their spawning migration if the genotype effect on AS persists across life-
520 stages (Clark *et al.* 2011; Eliason *et al.* 2011; Mottola *et al.* 2020). This may link aerobic
521 performance to demographic changes in salmon populations. It can potentially also be
522 relevant to survival of salmon after spawning, and thereby repeated spawning (iteroparity),
523 because iteroparity tendency is co-inherited with the same *vgll3* genotype as early maturation
524 (Aykanat *et al.* 2019).
525
526 Salmon males mature on average earlier than females (Fleming & Einum 2011) and the *vgll3*
527 locus is associated with freshwater maturation in male parr (Debes *et al.* 2021; Verta *et al.*
528 2020). Other studies in salmonids have found sex differences in the performance of 2+ year-
529 old or mature individuals, including a higher aerobic scope, but not SMR, and a higher cost
530 of swimming in males than in females (Clark *et al.* 2011; Makiguchi *et al.* 2017; Archer *et al.*
531 2020). The lack of sex differences in metabolic phenotypes in our study both across and
532 within age-at-maturity genotypes, therefore, supports the conclusion that sex-dependent life-
533 history variation is not reflected in metabolic rates during the juvenile stage, as suggested by
534 a few other studies (Regnier *et al.* 2015; Prokkola *et al.* 2021; Åsheim *et al.* 2021). Recently,
535 it was also shown that salmon females have a higher size threshold for maturation after one
536 year at sea compared to males (Tréhin *et al.* 2021), and further studies are required to link the
537 performance differences and sex-specific maturation schedules of salmon at sea.
538
539 Our experiment focussed on the genetic component determining age-at-maturity – rearing
540 fish until maturation was out of our scope, excluding the possibility to evaluate the
541 environmental component. The presence of genetic covariation between aerobic scope and
542 age-at-maturity constrains evolution because selection acting on either trait would alter the
543 phenotypic variation of the other (Lande 1979; Roff 1997). For example, natural selection
544 favouring later age-at-maturity would indirectly constrain the aerobic scope of juveniles to a

545 lower level, even if that may be a suboptimal phenotype. On the other hand, the genetic
546 covariation between aerobic scope at the juvenile stage and age-at-maturity may help
547 maintain optimal trait variation in age-at-maturity, e.g., by constraining the potentially
548 maladaptive environmentally induced variation in age-at-maturity (e.g., De Jong 1999; Tufto
549 2000). River geophysical properties are important determinants of the optimal age structure
550 of populations at maturity, whereby populations in smaller tributaries have a younger and
551 populations in large, fast-flowing rivers have an older age structure (Fleming & Einum 2011).
552 Therefore, forecasting the optimal age-at-maturity from juvenile phenotypic performance
553 (i.e., growth) would be maladaptive for salmon individuals if covariation between
554 performance traits and age at maturity was explained entirely by environmental effects.
555
556 Understanding the physiological basis of life-history variation in different life-stages and
557 environmental conditions can provide insights into the factors driving life-history evolution,
558 and hence, better predictions of the responses of populations to environmental changes. Wild
559 salmon populations have declined in recent decades, with a concomitant decrease in the
560 frequency of late maturing individuals in Arctic populations (Friedland *et al.* 2009; Czorlich
561 *et al.* 2018). Our study provides evidence that salmon that have a genetic tendency for late
562 maturation also have a genetic tendency for reduced aerobic scope in the juvenile stage
563 compared to salmon with a genetic tendency for early maturation, indicating that evolution
564 towards an earlier age-at-maturity can cause correlated selection towards increased aerobic
565 scope. In conclusion, our study emphasizes the value of better integration of aerobic scope
566 into theoretical predictions on life-history variation (Lailvaux & Husak 2014; Rosenfeld *et al.*
567 2020) and demonstrates Atlantic salmon as a genetically tractable system for addressing the
568 physiological and genetic relationships between traits underlying life-history variation.
569
570 **Data availability**
571 The data from metabolic rate measurements are available in Zenodo
572 (10.5281/zenodo.5153281). The R codes for SMR, MMR and statistical analysis are available
573 in an archived repository (v.1.0.0, doi: 10.5281/zenodo.5168998).
574
575 **Conflicts of interest**
576 The authors declare no conflicts of interest.
577
578 **Supplemental Material**

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

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