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

Short title: Genetic coupling of life-history and metabolism

Jenni M. Prokkola[1]*, Eirik R. Åsheim[1], Sergey Morozov[1], Paul Bangura[1], Jaakko Erkinaro[2], Annukka Ruokolainen[1], Craig R. Primmer[1,3], Tutku Aykanat[1]

[1]Organismal and Evolutionary Biology Research Programme, University of Helsinki, Finland
[2]Natural Resources Institute Finland
[3]Institute of Biotechnology, Helsinki Institute of Life Science (HiLIFE), University of Helsinki, Finland
*Corresponding author, PO Box 65, FI-00014 University of Helsinki, Finland, jenni.prokkola@helsinki.fi

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 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 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}

204 (allowing food to be available in the water column for a maximum amount of time while
205 minimizing the amount of leftover feed). Tanks were cleaned by scrubbing surfaces and
206 siphoning excess food once week^{-1} until June 1st, then approximately twice week^{-1} until
207 August 14th, then once week^{-1} until the end of the experiment. Mortality from first feeding in
208 March until PIT-tagging in July was approximately 8%. Water temperature during this time
209 increased from *ca.* 4.5 °C to 11°C (Fig. S1).
210
211 Between 14 and 21 July 2020, fish were captured by netting, anaesthetised with sodium
212 bicarbonate-buffered methanesulfonate (100 mg/L) and individually tagged with passive
213 integrated transponder (PIT) tags (length 8mm, width 1.2mm, Manruta, Quangdong, China)
214 inserted into abdominal cavity with a syringe and needle. Only fish that were >45mm were
215 tagged (Bangs *et al.* 2013). Afterwards, the fish were weighed, photographed (for a separate
216 study), and a small fin clip was collected from their caudal fin using a scalpel. Fish were
217 allowed to recover in aerated buckets briefly after anaesthesia, after which they were returned
218 to the rearing tanks. Total mortality due to tagging/anaesthesia was approx. 5%. After
219 tagging, environmental enrichment was provided to the tanks in the form of stones (diameter
220 approx. 8cm) placed in square, stainless-steel baskets (mesh size 2cm, width and length
221 20cm). Three baskets with three stones in each were provided to each tank.
222
223 The fin clip of each individual was placed in 20 µL of Lucigen QuickExtract DNA Extraction
224 Solution 1.0 and kept on ice or at -20°C until extractions were completed on the day of
225 sample collection according to manufacturer's instructions. DNA was stored at -20°C. DNA
226 was diluted 10-fold in water, after which genotyping was performed using a Kompetitive
227 Allele-Specific PCR (KASP, LGC genomics, UK) assay (He *et al.* 2014) for *vgll3* (Sinclair-
228 Waters *et al.* 2020) and *six6*, as well as for *sdY* to determine sex (details in online
229 supplemental material).
230
231 **Experimental design**
232 At minimum two weeks after PIT-tagging, fish from each family were divided into two tanks
233 at roughly even densities (mean 1.29 g L^{-1}, N = 145–152 tank^{-1}). The experiment (see Fig. 1,
234 created with BioRender.com) started in August 2020, at least three weeks after PIT-tagging
235 for each family. In the beginning of August, the relative age of the fish was approximately
236 2050 degree days, or 1313 Tau as calculated with the formula of Gorodilov (1996).

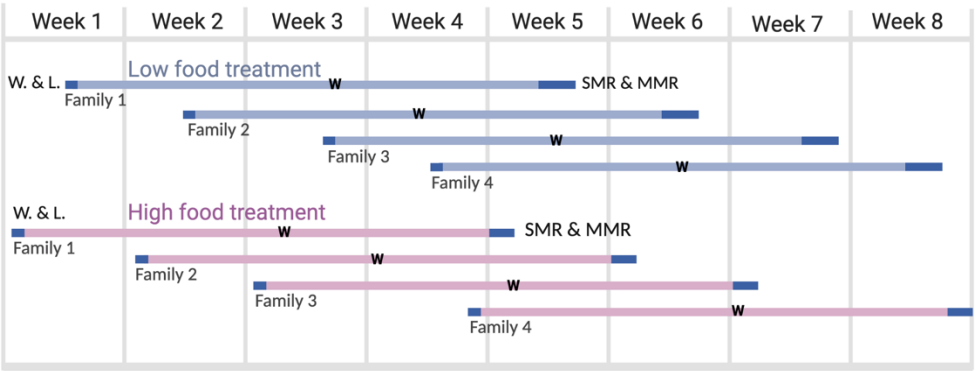


Fig. 1. Timeline of the experiment, starting 03 August 2020. The duration of low food and high food treatments was four weeks for each family. The "W" indicates when fish density of high food tanks was reduced to the level of low food tanks based on mean biomass. Each horizontal line represents a separate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

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

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

327 the spline-MMR data. For further details on SMR and MMR analyses, see online
328 supplementary information and data availability.
329
330 **Statistical analyses**
331 To test for the effects of treatment and genotype on metabolic variables, we ran separate
332 linear mixed models using SMR, MMR, and AS as response variables. This mixed model
333 framework allowed us to account for variation arising from i) fish body size, ii) family effects
334 (background genetic variation between families and tank effects), iii) timing of experiments
335 in relation to photoperiod and temperature during rearing, i.e., batch effects, iv) and technical
336 variation related to MMR measurements. Only individuals homozygous for both *vgll3* and
337 *six6* were included in the analysis because individuals heterozygous for either locus were not
338 measured from all families.
339
340 The response variables and body mass as a covariate were $\log_{10}$-transformed to account for
341 allometric scaling of metabolic rate. We included treatment, *vgll3* and *six6* genotypes, and
342 sex as fixed effects in all models. Random effects for SMR included family and measurement
343 batch. For MMR and AS they included family and the chamber where MMR was measured.
344 The chamber effect accounted for random variation among persons performing the chase
345 (half of the trials were consistently performed by the same person, J.P., the rest were divided
346 among three people), and variation due to the timing of the chase test (chamber order was
347 always the same when placing fish into chambers with only a few exceptions). Inclusion of
348 the name of person performing the chase as a random effect did not change the results of the
349 analysis or decrease the residual variance. Batch was not included as a random effect in
350 MMR and AS models due to model singularity. To test if genotype-specific metabolic rates
351 were affected by sex –because females mature later than males in both early and late age-at-
352 maturity genotypes– and by food availability, i.e., to test for genotype-by-environment
353 interaction, we fitted additional interactions into the models, including pairwise interactions
354 between *vgll3* and *six6* genotypes, between genotypes and treatment, and between genotypes
355 and sex. Further, the interaction of $\log_{10}$ body mass with treatment was included to test for
356 potential treatment-specific allometric scaling of metabolic rate, which may be related to
357 body composition differences between treatments. The full models were reduced by omitting
358 non-significant interactions in a stepwise process based on Type III test p-values to obtain
359 estimates for significant interaction effects. When interactions between fixed genotype effects
360 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).

393 **Maximum metabolic rate**

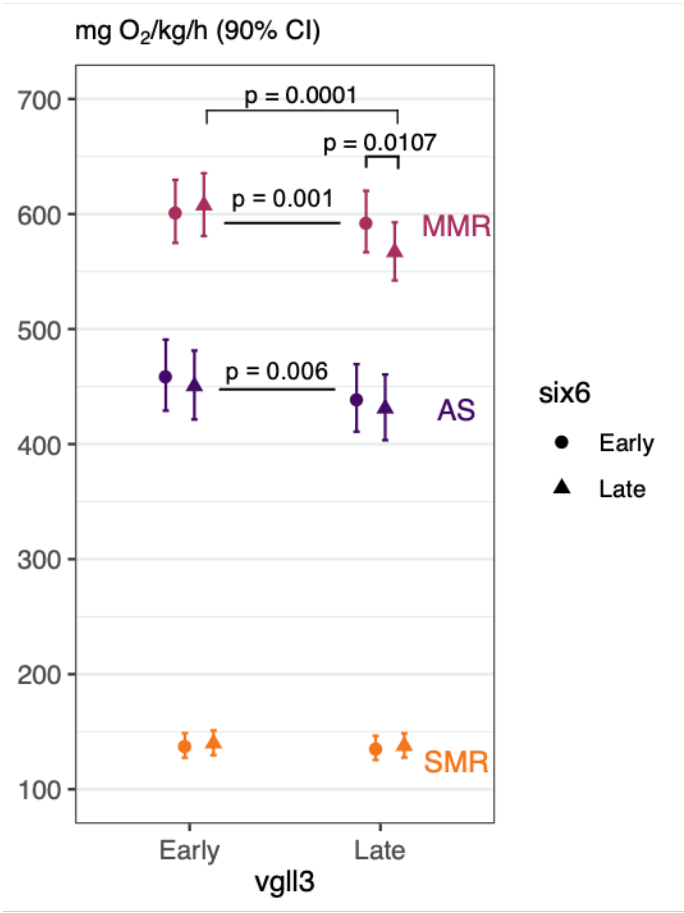
394 Fish with the *vgll3* early maturation genotype had a higher MMR than fish with the late
395 maturation genotype (Fig. 2, Table 1). *Vgll3* genotype also interacted with *six6*, such that
396 MMR was decreased when late maturation genotypes of the two loci cooccurred compared to
397 other genotype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

401

402 **Aerobic scope**

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

413



414

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

422

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					

423

424 **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).

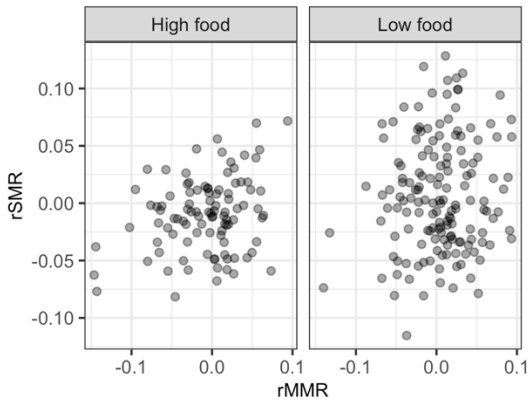


Fig 3. Scatter plots of family- and mass-corrected SMR and MMR in high and low food treatments. Dots represent individuals. Correlation coefficients are shown in Table 2. N = 100 (high food) and 153 (low food).

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

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

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

512 aerobic scope between *vgll3* early and late maturation genotypes may be reflected in the
513 resource acquisition and growth of juvenile salmon at elevated temperatures. Under these
514 conditions, higher aerobic scope may enable higher feeding capacity (Auer *et al.* 2015a),
515 because a temperature-related reduction in aerobic scope due to increased SMR and specific
516 dynamic action (the post-feeding increase in metabolic rate) could reduce appetite in fishes,
517 as is hypothesized in Jutfelt *et al.* (2021). Thus, our results indicate a potentially important
518 advantage for individuals carrying the early maturation genotype under global warming. This
519 advantage may also extend to higher survival of early maturing salmon relative to late
520 maturing salmon in their spawning migration if the genotype effect on AS persists across life-
521 stages (Clark *et al.* 2011; Eliason *et al.* 2011; Mottola *et al.* 2020). This may link aerobic
522 performance to demographic changes in salmon populations. It can potentially also be
523 relevant to survival of salmon after spawning, and thereby repeated spawning (iteroparity),
524 because iteroparity tendency is co-inherited with the same *vgll3* genotype as early maturation
525 (Aykanat *et al.* 2019).
526
527 Salmon males mature on average earlier than females (Fleming & Einum 2011) and the *vgll3*
528 locus is associated with freshwater maturation in male parr (Debes *et al.* 2021; Verta *et al.*
529 2020). Other studies in salmonids have found sex differences in the performance of 2+ year-
530 old or mature individuals, including a higher aerobic scope, but not SMR, and a higher cost
531 of swimming in males than in females (Clark *et al.* 2011; Makiguchi *et al.* 2017; Archer *et al.*
532 2020). The lack of sex differences in metabolic phenotypes in our study both across and
533 within age-at-maturity genotypes, therefore, supports the conclusion that sex-dependent life-
534 history variation is not reflected in metabolic rates during the juvenile stage, as suggested by
535 a few other studies (Regnier *et al.* 2015; Prokkola *et al.* 2021; Åsheim *et al.* 2021). Recently,
536 it was also shown that salmon females have a higher size threshold for maturation after one
537 year at sea compared to males (Tréhin *et al.* 2021), and further studies are required to link the
538 performance differences and sex-specific maturation schedules of salmon at sea.
539
540 Our experiment focussed on the genetic component determining age-at-maturity – rearing
541 fish until maturation was out of our scope, excluding the possibility to evaluate the
542 environmental component. The presence of genetic covariation between aerobic scope and
543 age-at-maturity constrains evolution because selection acting on either trait would alter the
544 phenotypic variation of the other (Lande 1979; Roff 1997). For example, natural selection
545 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.

References

Anttila, K. & Mänttäri, S. (2009) Ultrastructural differences and histochemical characteristics in swimming muscles between wild and reared Atlantic salmon. *Acta Physiologica,* **196,** 249-257.

Archer, L.C., Hutton, S.A., Harman, L., Poole, W.R., Gargan, P., McGinnity, P. & Reed, T.E. (2020) Metabolic traits in brown trout (*Salmo trutta*) vary in response to food restriction and intrinsic factors. *Conservation Physiology,* **8**, coaa096.

Armstrong, J.B. & Schindler, D.E. (2011) Excess digestive capacity in predators reflects a life of feast and famine. *Nature,* **476,** 84-87.

Arnott, S.A., Chiba, S. & Conover, D.O. (2006) Evolution of intrinsic growth rate: Metabolic costs drive trade-offs between growth and swimming performance in *Menidia menidia*. *Evolution,* **60,** 1269-1278.

Auer, S.K., Anderson, G.J., McKelvey, S., Bassar, R.D., McLennan, D., Armstrong, J.D., Nislow, K.H., Downie, H.K., McKelvey, L., Morgan, T.A.J., Salin, K., Orrell, D.L., Gauthey, A., Reid, T.C. & Metcalfe, N.B. (2018a) Nutrients from salmon parents alter selection pressures on their offspring. *Ecology Letters,* **21,** 287-295.

Auer, S.K., Bassar, R.D., Turek, D., Anderson, G.J., McKelvey, S., Armstrong, J.D., Nislow, K.H., Downie, H.K., Morgan, T.A.J., McLennan, D. & Metcalfe, N.B. (2020a) Metabolic rate interacts with resource availability to determine individual variation in microhabitat use in the wild. *American Naturalist,* **196,** 132-144.

Auer, S.K., Dick, C.A., Metcalfe, N.B. & Reznick, D.N. (2018b) Metabolic rate evolves rapidly and in parallel with the pace of life history. *Nature Communications,* **9**.

Auer, S.K., Salin, K., Anderson, G.J. & Metcalfe, N.B. (2015a) Aerobic scope explains individual variation in feeding capacity. *Biology Letters,* **11**.

Auer, S.K., Salin, K., Rudolf, A.M., Anderson, G.J. & Metcalfe, N.B. (2015b) Flexibility in metabolic rate confers a growth advantage under changing food availability. *Journal of Animal Ecology,* **84,** 1405-1411.

Auer, S.K., Salin, K., Rudolf, A.M., Anderson, G.J. & Metcalfe, N.B. (2015c) The optimal combination of standard metabolic rate and aerobic scope for somatic growth depends on food availability. *Functional Ecology,* **29,** 479-486.

Auer, S.K., Solowey, J.R., Rajesh, S. & Rezende, E.L. (2020b) Energetic mechanisms for coping with changes in resource availability. *Biology Letters,* **16**.

Aykanat, T., Ozerov, M., Vähä, J.P., Orell, P., Niemelä, E., Erkinaro, J. & Primmer, C.R. (2019) Co-inheritance of sea age at maturity and iteroparity in the Atlantic salmon *vgll3* genomic region. *Journal of Evolutionary Biology,* **32,** 343-355.

Aykanat, T., Rasmussen, M., Ozerov, M., Niemelä, E., Paulin, L., Vähä, J.P., Hindar, K., Wennevik, V., Pedersen, T., Svenning, M.A. & Primmer, C.R. (2020) Life-history genomic regions explain differences in Atlantic salmon marine diet specialization. *Journal of Animal Ecology,* **89,** 2677-2691.

Ayllon, F., Kjaerner-Semb, E., Furmanek, T., Wennevik, V., Solberg, M.F., Dahle, G., Taranger, G.L., Glover, K.A., Almen, M.S., Rubin, C.J., Edvardsen, R.B. & Wargelius, A. (2015) The *vgll3* locus controls age at maturity in wild and domesticated Atlantic salmon (*Salmo salar* L.) males. *PLoS Genetics,* **11**.

Barson, N.J., Aykanat, T., Hindar, K., Baranski, M., Bolstad, G.H., Fiske, P., Jacq, C., Jensen, A.J., Johnston, S.E., Karlsson, S., Kent, M., Oen, T.M., Niemelä, E., Nome, T., Naesje, T.F., Orell, P., Romakkaniemi, A., Saegrov, H., Urdal, K., Erkinaro, J., Lien, S. & Primmer, C.R. (2015) Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. *Nature,* **528,** 405-408.

Bates, D., Machler, M., Bolker, B.M. & Walker, S.C. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software,* **67,** 1-48.

Boratynski, Z., Koskela, E., Mappes, T. & Schroderus, E. (2013) Quantitative genetics and fitness effects of basal metabolism. *Evolutionary Ecology,* **27,** 301-314.

Brett, J.R. (1964) The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Research Board of Canada,* **21,** 1183-1226.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004) Toward a metabolic theory of ecology. *Ecology,* **85,** 1771-1789.

Burton, T., Killen, S.S., Armstrong, J.D. & Metcalfe, N.B. (2011) What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal Society B-Biological Sciences,* **278,** 3465-3473.

Careau, V., Thomas, D., Pelletier, F., Turki, L., Landry, F., Garant, D. & Réale, D. (2011) Genetic correlation between resting metabolic rate and exploratory behaviour in deer mice (*Peromyscus maniculatus*). *Journal of Evolutionary Biology,* **24,** 2153-2163.

Chabot, D., Steffensen, J.F. & Farrell, A.P. (2016) The determination of standard metabolic rate in fishes. *Journal of Fish Biology,* **88,** 81-121.

646 Chaput, G. (2012) Overview of the status of Atlantic salmon (*Salmo salar*) in the North
647 Atlantic and trends in marine mortality. *ICES Journal of Marine Science,* **69,** 1538-
648 1548.
649 Cheung, C.C., Thornton, J.E., Kuijper, J.L., Weigle, D.S., Clifton, D.K. & Steiner, R.A.
650 (1997) Leptin is a metabolic gate for the onset of puberty in the female rat.
651 *Endocrinology,* **138,** 855-858.
652 Cheverud, J.M. & Routman, E.J. (1995) Epistasis and its contribution to genetic variance
653 components. *Genetics,* **139,** 1455-1461.
654 Cheverud, J.M. & Routman, E.J. (1996) Epistasis as a source of increased additive genetic
655 variance at population bottlenecks. *Evolution,* **50,** 1042-1051.
656 Clark, T.D., Jeffries, K.M., Hinch, S.G. & Farrell, A.P. (2011) Exceptional aerobic scope and
657 cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie
658 resilience in a warming climate. *Journal of Experimental Biology,* **214,** 3074-3081.
659 Czorlich, Y., Aykanat, T., Erkinaro, J., Orell, P. & Primmer, C.R. (2018) Rapid sex-specific
660 evolution of age at maturity is shaped by genetic architecture in Atlantic salmon.
661 *Nature Ecology & Evolution,* **2,** 1800-1807.
662 Czorlich, Y., Aykanat, T., Erkinaro, J., Orell, P. & Primmer, C.R. (2021) Evolution in salmon
663 life-history induced by direct and indirect effects of fishing. *bioRxiv*
664 2021.01.08.425869.
665 Dammhahn, M., Dingemanse, N.J., Niemelä, P.T. & Réale, D. (2018) Pace-of-life
666 syndromes: a framework for the adaptive integration of behaviour, physiology and life
667 history. *Behavioral Ecology and Sociobiology,* **72**, 62.
668 De Jong, G. (1999) Unpredictable selection in a structured population leads to local genetic
669 differentiation in evolved reaction norms. *Journal of Evolutionary Biology,* **12,** 839-
670 851.
671 Debat, V. & David, P. (2001) Mapping phenotypes: canalization, plasticity and
672 developmental stability. *Trends in Ecology & Evolution,* **16,** 555-561.
673 Debes, P.V., Piavchenko, N., Ruokolainen, A., Ovaskainen, O., Moustakas-Verho, J.E.,
674 Parre, N., Aykanat, T., Erkinaro, J. & Primmer, C.R. (2021) Polygenic and major-
675 locus contributions to sexual maturation timing in Atlantic salmon. *Molecular*
676 *Ecology*. Early online.
677 Eliason, E.J., Clark, T.D., Hague, M.J., Hanson, L.M., Gallagher, Z.S., Jeffries, K.M., Gale,
678 M.K., Patterson, D.A., Hinch, S.G. & Farrell, A.P. (2011) Differences in thermal
679 tolerance among sockeye salmon populations. *Science,* **332,** 109-112.

Eliason, E.J., Wilson, S.M., Farrell, A.P., Cooke, S.J. & Hinch, S.G. (2013) Low cardiac and aerobic scope in a coastal population of sockeye salmon *Oncorhynchus nerka* with a short upriver migration. *Journal of Fish Biology,* **82,** 2104-2112.

Elliott, J.M. & Hurley, M.A. (1997) A functional model for maximum growth of Atlantic Salmon parr, *Salmo salar*, from two populations in northwest England. *Functional Ecology,* **11,** 592-603.

Farrell, A.P. (2009) Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. *Journal of Experimental Biology,* **212,** 3771-3780.

Fleming, I.A. & Einum, S. (2011) Reproductive ecology: A tale of two sexes. *In:* Aas et al. *Atlantic Salmon Ecology*, pp. 33–65. Wiley-Blackwell.

Forstner, H. (1983) An automated multiple-chamber intermittent-flow respirometer. pp. 111-126. Springer Berlin Heidelberg, Berlin, Heidelberg.

Friedland, K.D., MacLean, J.C., Hansen, L.P., Peyronnet, A.J., Karlsson, L., Reddin, D.G., Maoileidigh, N.O. & McCarthy, J.L. (2009) The recruitment of Atlantic salmon in Europe. *ICES Journal of Marine Science,* **66,** 289-304.

Frisch, R.E. (1985) Fatness, menarche, and female fertility. *Perspectives in Biology and Medicine,* **28,** 611-633.

Fry, F. E. J. (1947) Effects of the environment on animal activity. Ontario Fisheries Research Laboratory Publication, Biol. Ser. 1-62.

Gamperl, A.K. & Farrell, A.P. (2004) Cardiac plasticity in fishes: environmental influences and intraspecific differences. *Journal of Experimental Biology,* **207,** 2539-2550.

Gillespie, J.H. & Turelli, M. (1989) Genotype-environment interactions and the maintenance of polygenic variation. *Genetics,* **121,** 129-138.

Gorodilov, Y.N. (1996) Description of the early ontogeny of the Atlantic salmon, *Salmo salar*, with a novel system of interval (state) identification. *Environmental Biology of Fishes* **47**, 109-127.

Gutteling, E.W., Doroszuk, A., Riksen, J.A.G., Prokop, Z., Reszka, J. & Kammenga, J.E. (2007) Environmental influence on the genetic correlations between life-history traits in *Caenorhabditis elegans*. *Heredity,* **98,** 206-213.

Harianto, J., Carey, N. & Byrne, M. (2019) respR-An R package for the manipulation and analysis of respirometry data. *Methods in Ecology and Evolution,* **10,** 912-920.

712 Harter, T.S., Zanuzzo, F.S., Supuran, C.T., Gamperl, A.K. & Brauner, C.J. (2019) Functional
713 support for a novel mechanism that enhances tissue oxygen extraction in a teleost fish.
714 *Proceedings of the Royal Society B-Biological Sciences,* **286**, 20190339.
715 He, C.L., Holme, J. & Anthony, J. (2014) SNP Genotyping: The KASP Assay. *Crop*
716 *Breeding: Methods and Protocols,* **1145,** 75-86.
717 Hendry, A.P., Berg, O.K. & Quinn, T.P. (1999) Condition dependence and adaptation-by-
718 time: breeding date, life history, and energy allocation within a population of salmon.
719 *Oikos,* **85,** 499-514.
720 Hughes, K.A., Rodd, F.H. & Reznick, D.N. (2005) Genetic and environmental effects on
721 secondary sex traits in guppies (*Poecilia reticulata*). *Journal of Evolutionary Biology,*
722 **18,** 35-45.
723 Hutchings, J.A. & Jones, M.E.B. (1998) Life history variation and growth rate thresholds for
724 maturity in Atlantic salmon, *Salmo salar*. *Canadian Journal of Fisheries and Aquatic*
725 *Sciences,* **55,** 22-47.
726 Johnston, S.E., Orell, P., Pritchard, V.L., Kent, M.P., Lien, S., Niemelä, E., Erkinaro, J. &
727 Primmer, C.R. (2014) Genome-wide SNP analysis reveals a genetic basis for sea-age
728 variation in a wild population of Atlantic salmon (*Salmo salar*). *Molecular Ecology,*
729 **23,** 3452-3468.
730 Jutfelt, F., Norin, T., Åsheim, E.R., Rowsey, L.E., Andreassen, A.H., Morgan, R., Clark, T.D.
731 & Speers-Roesch, B. (2021) 'Aerobic scope protection' reduces ectotherm growth
732 under warming. *Functional Ecology. Early online.*
733 Killen, S., Christensen, E., Cortese, D., Zavorka, L., Norin, T., Cotgrove, L., Crespel, A.,
734 Munson, A., Nati, J., Papatheodoulou, M. & McKenzie, D. (2021) Guidelines for
735 reporting methods to estimate metabolic rates by aquatic intermittent-flow
736 respirometry. *EcoEvoRxiv*. ecoevorxiv.org/gnzh7
737 Killen, S.S., Glazier, D.S., Rezende, E.L., Clark, T.D., Atkinson, D., Willener, A.S.T. &
738 Halsey, L.G. (2016) Ecological influences and morphological correlates of resting and
739 maximal metabolic rates across teleost fish species. *American Naturalist,* **187,** 592-
740 606.
741 Killen, S.S., Marras, S. & McKenzie, D.J. (2011) Fuel, fasting, fear: routine metabolic rate
742 and food deprivation exert synergistic effects on risk-taking in individual juvenile
743 European sea bass. *Journal of Animal Ecology,* **80,** 1024-1033.
744 Krams, I.A., Niemelä, P.T., Trakimas, G., Krams, R., Burghardt, G.M., Krama, T., Kuusik,
745 A., Mand, M., Rantala, M.J., Mand, R., Kekälainen, J., Sirkka, I., Luoto, S. & Kortet,

R. (2017) Metabolic rate associates with, but does not generate covariation between, behaviours in western stutter-trilling crickets, *Gryllus integer*. *Proceedings of the Royal Society B-Biological Sciences,* **284**, 20162481.

Kurko, J., Debes, P.V., House, A.H., Aykanat, T., Erkinaro, J. & Primmer, C.R. (2020) Transcription profiles of age-at-maturity-associated genes suggest cell fate commitment regulation as a key factor in the Atlantic salmon maturation process. *G3-Genes Genomes Genetics,* **10,** 235-246.

Lailvaux, S.P. & Husak, J.F. (2014) The life-history of whole-organism performance. *Quarterly Review of Biology,* **89,** 285-318.

Lande, R. (1979) Quantitative genetic analysis of multivariate evolution, applied to brain-body size allometry. *Evolution,* **33,** 402-416.

Lenth, R. V. (2020) emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.3.

Little, A.G., Dressler, T., Kraskura, K., Hardison, E., Hendriks, B., Prystay, T., Farrell, A.P., Cooke, S.J., Patterson, D.A., Hinch, S.G. & Eliason, E.J. (2020) Maxed out: optimizing accuracy, precision, and power for field measures of maximum metabolic rate in fishes. *Physiological and Biochemical Zoology,* **93,** 243-254.

Long, J.A. (2019) interactions: Comprehensive, User-Friendly Toolkit for Probing Interactions. R package version 1.1.0, https://cran.r-project.org/package=interactions.

Lüdecke, D. (2018). ggeffects: Tidy data frames of marginal effects from regression models. *Journal of Open Source Software* 3, 772.

Maiti, U., Sadowska, E.T., Chrzascik, K.M. & Koteja, P. (2019) Experimental evolution of personality traits: open-field exploration in bank voles from a multidirectional selection experiment. *Current Zoology,* **65,** 375-384.

Makiguchi, Y., Nii, H., Nakao, K. & Ueda, H. (2017) Sex differences in metabolic rate and swimming performance in pink salmon (*Oncorhynchus gorbuscha*): the effect of male secondary sexual traits. *Ecology of Freshwater Fish,* **26,** 322-332.

Mathot, K.J., Dingemanse, N.J. & Nakagawa, S. (2019) The covariance between metabolic rate and behaviour varies across behaviours and thermal types: meta-analytic insights. *Biological Reviews,* **94,** 1056-1074.

McArley, T.J., Morgenroth, D., Zena, L.A., Ekström, A.E., Sandblom, E. (2021) Normoxic limitation of maximal oxygen consumption rate, aerobic scope and cardiac performance in exhaustively exercised rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology*, jeb.242614.

780 Merilä, J. & Sheldon, B.C. (1999) Genetic architecture of fitness and nonfitness traits:
781 empirical patterns and development of ideas. *Heredity,* **83,** 103-109.
782 Metcalfe, N.B., Taylor, A.C. & Thorpe, J.E. (1995) Metabolic rate, social status and life-
783 history strategies in Atlantic salmon. *Animal Behaviour,* **49,** 431-436.
784 Metcalfe, N.B., Van Leeuwen, T.E. & Killen, S.S. (2016) Does individual variation in
785 metabolic phenotype predict fish behaviour and performance? *Journal of Fish*
786 *Biology,* **88,** 298-321.
787 Mobley, K.B., Aykanat, T., Czorlich, Y., House, A., Kurko, J., Miettinen, A., Moustakas-
788 Verho, J., Salgado, A., Sinclair-Waters, M., Verta, J.P. & Primmer, C.R. Maturation
789 in Atlantic salmon (*Salmo salar*, Salmonidae): a synthesis of ecological, genetic, and
790 molecular processes. *Reviews in Fish Biology and Fisheries* **31**, 523-571.
791 Moghadam, H.K., Poissant, J., Fotherby, H., Haidle, L., Ferguson, M.M. & Danzmann, R.G.
792 (2007) Quantitative trait loci for body weight, condition factor and age at sexual
793 maturation in Arctic charr (*Salvelinus alpinus*): comparative analysis with rainbow
794 trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Molecular Genetics*
795 *and Genomics,* **277,** 647-661.
796 Morozov, S., McCairns, R.J.S. & Merilä, J. (2019) FishResp: R package and GUI application
797 for analysis of aquatic respirometry data. *Conservation Physiology,* **7**, coz003.
798 Mottola, G., Kristensen, T. & Anttila, K. (2020) Compromised thermal tolerance of
799 cardiovascular capacity in upstream migrating Arctic char and brown trout-are hot
800 summers threatening migrating salmonids? *Conservation Physiology,* **8**, coaa101.
801 Moustakas-Verho, J.E., Kurko, J., House, A.H., Erkinaro, J., Debes, P. & Primmer, C.R.
802 (2020) Developmental expression patterns of six6: A gene linked with spawning
803 ecotypes in Atlantic salmon. *Gene Expression Patterns,* **38**, 119149.
804 Niemelä, P.T. & Dingemanse, N.J. (2018) Meta-analysis reveals weak associations between
805 intrinsic state and personality. *Proceedings of the Royal Society B-Biological*
806 *Sciences,* **285**, 20172823.
807 Niitepõld, K. (2010) Genotype by temperature interactions in the metabolic rate of the
808 Glanville fritillary butterfly. *Journal of Experimental Biology,* **213,** 1042-1048.
809 Nikinmaa, M., Berenbrink, M. & Brauner, C.J. (2019) Regulation of erythrocyte function:
810 Multiple evolutionary solutions for respiratory gas transport and its regulation in fish.
811 *Acta Physiologica,* **227**, e13299.
812 Norin, T. & Clark, T.D. (2016) Measurement and relevance of maximum metabolic rate in
813 fishes. *Journal of Fish Biology,* **88,** 122-151.

Norin, T. & Malte, H. (2012) Intraspecific variation in aerobic metabolic rate of fish: relations with organ size and enzyme activity in brown trout. *Physiological and Biochemical Zoology,* **85,** 645-656.

O'Connor, K.I., Taylor, A.C. & Metcalfe, N.B. (2000) The stability of standard metabolic rate during a period of food deprivation in juvenile Atlantic salmon. *Journal of Fish Biology,* **57,** 41-51.

Polverino, G., Santostefano, F., Diaz-Gil, C. & Mehner, T. (2018) Ecological conditions drive pace-of-life syndromes by shaping relationships between life history, physiology and behaviour in two populations of Eastern mosquitofish. *Scientific Reports,* **8**, 14673.

Priede, I.G. (1985) Metabolic scope in fishes. *In*: Tytler P., Calow P. (eds) Fish energetics. Springer, Dordrecht.

Pritchard, V.L., Mäkinen, H., Vähä, J.P., Erkinaro, J., Orell, P. & Primmer, C.R. (2018) Genomic signatures of fine-scale local selection in Atlantic salmon suggest involvement of sexual maturation, energy homeostasis and immune defence-related genes. *Molecular Ecology,* **27,** 2560-2575.

Prokkola, J.M., Alioravainen, N., Mehtätalo, L., Hyvärinen, P., Lemopoulos, A., Metso, S. & Vainikka, A. (2021) Does parental angling selection affect the behavior or metabolism of brown trout parr? *Ecology and Evolution,* **11,** 2630-2644.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raby, G.D., Doherty, C.L.J., Mokdad, A., Pitcher, T.E. & Fisk, A.T. (2020) Post-exercise respirometry underestimates maximum metabolic rate in juvenile salmon. *Conservation Physiology,* **8**, coaa063.

Regnier, T., Labonne, J., Chat, J., Yano, A., Guiguen, Y. & Bolliet, V. (2015) No early gender effects on energetic status and life history in a salmonid. *Royal Society Open Science,* **2**, 150441.

Ricklefs, R.E. & Wikelski, M. (2002) The physiology/life-history nexus. *Trends in Ecology & Evolution,* **17,** 462-468.

Roff, D. A. (1997) Evolutionary quantitative genetics. Chapman and Hall, New York.

Roff, D.A. (1994) The evolution of dimorphic traits – Predicting the genetic correlation between environments. *Genetics,* **136,** 395-401.

Rosenfeld, J., Richards, J., Allen, D., Van Leeuwen, T. & Monnet, G. (2020) Adaptive trade-offs in fish energetics and physiology: insights from adaptive differentiation among

juvenile salmonids. *Canadian Journal of Fisheries and Aquatic Sciences,* **77,** 1243-1255.

Rosenfeld, J., Van Leeuwen, T., Richards, J. & Allen, D. (2015) Relationship between growth and standard metabolic rate: measurement artefacts and implications for habitat use and life-history adaptation in salmonids. *Journal of Animal Ecology,* **84,** 4-20.

Salminen, M. (1997) Relationships between smolt size, postsmolt growth and sea age at maturity in Atlantic salmon ranched in the Baltic Sea. *Journal of Applied Ichthyology-Zeitschrift Fur Angewandte Ichthyologie,* **13,** 121-130.

Sandblom, E., Clark, T.D., Gräns, A., Ekström, A., Brijs, J., Sundström, L.F., Odelström, A., Adill, A., Aho, T. & Jutfelt, F. (2016) Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. *Nature Communications,* **7**, 11447.

Sinclair-Waters, M., Odegard, J., Korsvoll, S.A., Moen, T., Lien, S., Primmer, C.R. & Barson, N.J. (2020) Beyond large-effect loci: large-scale GWAS reveals a mixed large-effect and polygenic architecture for age at maturity of Atlantic salmon. *Genetics Selection Evolution,* **52**, 9.

Skilbrei, O.T. (1989) Relationship between smolt length and growth and maturation in the sea of individually tagged Atlantic salmon (*Salmo salar*). *Aquaculture,* **83,** 95-108.

Stearns, S.C. (2000) Life history evolution: successes, limitations, and prospects. *Naturwissenschaften,* **87,** 476-486.

Svendsen, M.B.S., Bushnell, P.G. & Steffensen, J.F. (2016) Design and setup of intermittent-flow respirometry system for aquatic organisms. *Journal of Fish Biology,* **88,** 26-50.

Taranger, G.L., Carrillo, M., Schulz, R.W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F.A., Dufour, S., Karlsen, O., Norberg, B., Andersson, E. & Hausen, T. (2010) Control of puberty in farmed fish. *General and Comparative Endocrinology,* **165,** 483-515.

Theriault, V., Garant, D., Bernatchez, L. & Dodson, J.J. (2007) Heritability of life-history tactics and genetic correlation with body size in a natural population of brook charr (*Salvelinus fontinalis*). *Journal of Evolutionary Biology,* **20,** 2266-2277.

Thorpe, J.E. (2007) Maturation responses of salmonids to changing developmental opportunities. *Marine Ecology Progress Series,* **335,** 285-288.

Thorpe, J.E., Mangel, M., Metcalfe, N.B. & Huntingford, F.A. (1998) Modelling the proximate basis of salmonid life-history variation, with application to Atlantic salmon, *Salmo salar* L. *Evolutionary Ecology,* **12,** 581-599.

Tréhin, C., Rivot, E., Lamireau, L., Meslier, L., Besnard, A.-L., Gregory, S.D. & Nevoux, M. (2021) Growth during the first summer at sea modulates sex-specific maturation schedule in Atlantic salmon. *Canadian Journal of Fisheries and Aquatic Sciences,* **78,** 659-669.

Tufto, J. (2000) The evolution of plasticity and nonplastic spatial and temporal adaptations in the presence of imperfect environmental cues. *American Naturalist,* **156,** 121-130.

Verta, J.P., Debes, P.V., Piavchenko, N., Ruokolainen, A., Ovaskainen, O., Moustakas-Verho, J.E., Tillanen, S., Parre, N., Aykanat, T., Erkinaro, J. & Primmer, C.R. (2020) Cis-regulatory differences in isoform expression associate with life history strategy variation in Atlantic salmon. *PLoS Genetics,* **16**, e1009055.

Ward, A.J.W., Webster, M.M. & Hart, P.J.B. (2006) Intraspecific food competition in fishes. *Fish and Fisheries,* **7,** 231-261.

Weimerskirch, H. (1992) Reproductive effort in long-lived birds – Age-specific patterns of condition, reproduction and survival in the wandering albatross. *Oikos,* **64,** 464-473.

Wickham, H. (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Zeng, L.Q., Zhang, A.J., Killen, S.S., Cao, Z.D., Wang, Y.X. & Fu, S.J. (2017) Standard metabolic rate predicts growth trajectory of juvenile Chinese crucian carp (*Carassius auratus*) under changing food availability. *Biology Open,* **6,** 1305-1309.

Zueva, K.J., Lumme, J., Veselov, A.E., Primmer, C.R. & Pritchard, V.L. (2021) Population genomics reveals repeated signals of adaptive divergence in the Atlantic salmon of north-eastern Europe. *Journal of Evolutionary Biology,* **34,** 866-878.

Åsheim, E. R., Prokkola, J. M., Morozov, S., Aykanat, T., Primmer, C. R. Standard metabolic rate does not associate with age-at-maturity genotype in juvenile Atlantic salmon. *bioRxiv,* https://doi.org/10.1101/2021.08.25.457673.