



HAL
open science

Growth regulation in brook charr *Salvelinus fontinalis*

Maria Angelica Martinez-Silva, Aurélie Dupont-Prinet, Carolyne Houle, Marie Vagner, Dany Garant, Louis Bernatchez, Céline Audet

► **To cite this version:**

Maria Angelica Martinez-Silva, Aurélie Dupont-Prinet, Carolyne Houle, Marie Vagner, Dany Garant, et al.. Growth regulation in brook charr *Salvelinus fontinalis*. *General and Comparative Endocrinology*, 2023, 331, pp.114160. 10.1016/j.ygcen.2022.114160 . hal-03856976

HAL Id: hal-03856976

<https://hal.science/hal-03856976v1>

Submitted on 17 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Growth regulation in brook charr *Salvelinus fontinalis***

2
3 Martínez-Silva Maria A.¹, Dupont-Prinet Aurélie¹, Houle Carolyne², Vagner Marie³, Garant
4 Dany², Bernatchez Louis⁴, Audet Céline¹

5
6 ¹Institut des sciences de la mer de Rimouski, Université du Québec à Rimouski, Rimouski, QC,
7 G5L 3A1, Canada

8 ²Département de biologie, Université du Sherbrooke, Sherbrooke, QC, J1K 2R1, Canada

9 ³Laboratoire des Sciences de l'Environnement Marin, UMR 6539 (CNRS/Univ
10 Brest/IRD/Ifremer), Plouzané, 29280, France

11 ⁴Institut de Biologie Intégrative et des Systèmes (IBIS), Département de Biologie, Université du
12 Laval, Québec, QC, G1V 0A6, Canada

13
14 Corresponding author: Maria Angelica Martínez-Silva, Institut des sciences de la mer de Rimouski,
15 Université du Québec à Rimouski, 310 allée des Ursulines, Rimouski, QC, Canada, G5L 3A1,
16 mariaangelica.martinezsilva@uqar.ca

17

18

19 **ABSTRACT**

20 Fish growth can be modulated through genetic selection. However, it is not known whether
21 growth regulatory mechanisms modulated by genetic selection can provide information about
22 phenotypic growth variations in families or populations. Following a five-generation breeding
23 program conducted in our lab that selected for the absence of early sexual maturity and increased
24 growth in brook charr, we examined the impact of selection, **family performance**, and **individual**
25 **phenotype** on growth regulation pathways at the molecular level in the brain, pituitary, liver, and

26 muscle. At age 1+, individuals from four of the highest performing and four of the lowest
27 performing families in terms of growth were sampled in each line (control and selected). The gene
28 expression levels of three reference and ten target genes were analyzed by real-time PCR. Results
29 showed that better growth performance (in terms of weight and length at age) in the selected line
30 was associated with an upregulation in the expression of genes involved in the growth hormone
31 (GH)/insulin growth factor-1 (IGF-1) axis, including the *igf-1* receptor in pituitary; the *gh-1*
32 receptor and *igf-1* in liver; and *ghr* and *igf-1r* in white muscle. When looking at gene expression
33 within families, family performance and individual phenotypes were associated with upregulations
34 of the *leptin* receptor and *neuropeptid Y*—genes related to appetite regulation—in the slower-
35 growing phenotypes. However, other genes related to appetite (*ghrelin*, *somatostatin*) or involved
36 in muscle growth (*myosin heavy chain*, *myogenin*) were not differentially expressed. This study
37 highlights how transcriptomics may improve our understanding of the roles of different key
38 endocrine steps that regulate physiological performance. Large variations in growth still exist in
39 the selected line, indicating that the full genetic selection potential has not been reached.

40

41 **Keywords:** Selection, Phenotype, Growth, Transcriptomics, *gh/igf-1* axis, Appetite regulation

42

43

44 1. INTRODUCTION

45 Fish reproductive success in nature is determined not only by the number of offspring that
46 an individual produces, but also by how many offspring survive to reproductive maturity (Clutton-
47 Brock, 1988). Large males, which may be preferred by females, can dominate competitors in

48 contests for mates or breeding territories, and large females can produce more and larger offspring
49 than small ones (Perry *et al.*, 2005; Anderson *et al.*, 2010). Aquaculture production aims to produce
50 large fish that would invest in growth instead of reproduction and that would best adapt to the
51 captive environment (Gjedrem, 2005; Sauvage *et al.*, 2010; Bastien *et al.*, 2011). Selective breeding
52 exploits the substantial genetic variation that is present for desirable traits. Thus, a high growth rate
53 as well as the absence of early sexual maturity are the most used criteria since energy is
54 preferentially invested in growth rather than in gamete production (e.g., Nilsson, 1990; Bastien *et*
55 *al.*, 2011).

56 Growth in teleosts is controlled at the endocrine level, mainly by the growth hormone (GH)
57 / insulin factor 1 (IGF-1) axis (Björnsson, 1997; Wood *et al.*, 2005; Duan *et al.*, 2010; Vélez *et al.*,
58 2017). GH, which is synthesized by the pituitary gland, regulates many functions, including
59 somatic growth, energy metabolism, reproduction, digestion, osmoregulation, and immune
60 function (Kawaguchi *et al.*, 2013). The release and synthesis of GH in the brain and peripheral
61 tissues are i) stimulated by neuroendocrine factors, such as neuropeptide Y (NPY) (Aldegunde and
62 Mancebo, 2006) and ghrelin (GRL) (Rønnestad, *et al.*, 2017; Perelló-Amorós *et al.*, 2018), and ii)
63 inhibited by somatostatin (SRIF, mainly synthesized in the brain) (Nelson and Sheridan, 2005;
64 Very and Sheridan, 2007; Sheridan and Hagemester, 2010; Volkoff *et al.*, 2010). GH stimulates
65 the production of liver IGF-1 (Volkoff *et al.*, 2010), so its physiological effects are usually indirect,
66 via IGF-1 actions. Appetite regulation is another key process controlling growth and is regulated
67 by hormones also acting as appetite stimulators (i.e., orexigenic factors NPY and GRL) (Breton *et*
68 *al.*, 1989; Cerdá-Reverter and Larhammar, 2000; Rønnestad, *et al.*, 2017) or appetite inhibitors
69 (i.e., anorectic factor: leptin LEP) (Hoskins and Volkoff, 2012; Dar *et al.*, 2018). The continued
70 production of muscle fibres is another important process that controls fish growth (Ahammad *et*

71 *al.*, 2015). Teleosts are unique among vertebrates because of their continued growth due to the
72 continuous production of muscle fibres from birth to death (Ahammad *et al.*, 2015). Some key
73 myofibrillar proteins such as myosin, actin, tropomyosin, and troponin are specifically expressed
74 in muscle tissue and are involved in its contraction (Skaara and Regenstein, 1990; Zhang *et al.*,
75 2011). Other myogenic factors involved in tissue differentiation and maturation processes, such as
76 myosin heavy-chain (MHC) and myogenic regulatory factor (MRF4), are key for understanding
77 growth-regulating mechanisms (Vélez *et al.*, 2016).

78 Few studies have focused on how the selection process affects growth regulation in brook
79 charr *Salvelinus fontinalis*. Sauvage *et al.* (2010) reported that selective breeding led to a 4.16%
80 difference in expressed genes between the control and domesticated lines at the juvenile stage. In
81 particular, they observed that genes involved in growth pathways (e.g., transforming growth factor
82 b and T complex protein 1) were generally more highly expressed in the selected line than in the
83 control line. Studies in other salmonids, such *Oncorhynchus kisutch* and *O. mykiss*, evaluated the
84 effects of domestication on growth (Devlin *et al.*, 2009; Tymchuk *et al.*, 2009) and the relationship
85 between genomics and selection in aquaculture based on the study of divergence and genome size
86 (Hessen *et al.*, 2010; Pankova *et al.*, 2017). However, no one has looked at the growth regulation
87 pathways occurring in different brook charr tissues, and that is the objective of the study presented
88 here.

89 Selective breeding is particularly well-developed for brook charr, for which several studies
90 have been carried out with both anadromous (seawater migratory) and resident fish (Laval strain,
91 Québec). Perry *et al.* (2004) showed that higher fertility was associated with higher mean fry
92 length, suggesting that stabilizing selection for juvenile length occurred prior to yolk sac resorption.
93 Furthermore, parental-based genetic variance for early size traits appears to be partially segregated

94 at the embryo–fry boundary, with maternal genetic variance being high prior to yolk-sac resorption
95 and relatively low thereafter (Perry *et al.*, 2004, 2005). Significant heritability for traits related to
96 the accumulation and use of energy reserves was found in two out of three different strains used
97 by the Québec fish-farming industry, which include the Laval strain (Crespel *et al.*, 2013).
98 Domestication has led to large increases in fish weight: for *S. fontinalis* from the same Laval strain,
99 the weight of selected fish at age 22 months increased by 23% between the F1 and F2 generations
100 and by 32% from F2 to F3 (Bastien *et al.*, 2011).

101 The main goal of this study was to understand how the genetic selection process modifies
102 the growth regulatory pathway of brook charr at the molecular level. To achieve this, we had three
103 different objectives: 1) looking for regulation of growth traits between lines—one under selection,
104 the other not, 2) looking within each selected and control lines, for among-family differences- in
105 average growth phenotypes, which we termed family performance, and 3) looking within families
106 of each lines, at individuals ~~within families~~ that expressed extreme growth phenotypes, which we
107 termed slow- and fast-growing. We tested the hypothesis that selection enhanced the differential
108 expressions of genes involved in the GH/IGF-1 axis and in appetite control as well as in muscle
109 growth between slow- and fast-growing phenotypes and family performance. We also wanted to
110 find molecular indicators that could be implemented in a selection program to enhance sustainable
111 production for brook charr aquaculture.

112

113 2. METHODOLOGY

114 2.1. Selection process

115 A selective breeding program described by Bastien *et al.* (2011) and Sauvage *et al.* (2010)
116 was initiated in 1994 using wild *S. fontinalis* from the Laval River (Québec; 48.449° N, 68.059°
117 W). Briefly, a combined between- and within-family selection protocol was applied based on 1)
118 the absence of precocious sexual maturation at 22 months and 2) growth performance in sexually
119 immature fish. A control line was created by the arbitrary selection of equal numbers of fish from
120 each family for every generation (i.e., domestication to culture conditions, but no intentional
121 selection) (Bastien *et al.* 2011). This line was maintained over the same period, which allowed us
122 to perform studies aimed at tracking temporal genetic and phenotypic changes occurring in selected
123 vs. control ~~strains~~ lines reared in the exact same environment. It is important to note that this control
124 group—even if it was not selected by the criteria of growth and absence of maturity—can be
125 considered as domesticated across generations (non-directed selection). Fish were healthy
126 throughout this study, and we encountered no problems in maintaining all families and lines.

127

128 2.2. Rearing conditions

129 Fertilized eggs were incubated in darkness. Each family was incubated separately in
130 individual trays with screened bottoms that allowed the upwelling of water through the egg layers
131 during incubation and the inflow from the upstream side during fry rearing. Water temperature
132 followed the natural winter decrease but was not allowed to drop below 4°C. At hatching,
133 temperature was gradually increased by 1°C per week to reach 8°C, providing optimal conditions

Commenté [MV1]: Ne vaut il pas mieux garder le meme
terme partout ?
A vérifier partout ailleurs ?

134 for first feeding. At the beginning of June, when natural water conditions reached 8°C, no further
135 temperature adjustments were made, and fish were reared under natural temperature and
136 photoperiod conditions in flow-through dechlorinated fresh water in our wet lab facilities (maximal
137 temperature: 15°C in September; minimal temperature 3°C in February). Each family was
138 maintained in its individual tray until fish reached a size that allowed family identification by fin
139 clippings (eight possible marks combining adipose, right and left pelvic fins). Families (from both
140 control and selected combined lines) with different markings were randomly pooled in five 250 L
141 rearing tanks and later then in five 500 L tanks at the next measurement period (i.e. combien de
142 temps après?). In general, fish from six families were placed in each 250 L tank (400 per family,
143 2400 ind/tank, mean charge of 9.2 kg/m³), and then in 500 L tanks (200 per family; 1200 ind/tank,
144 mean charge of 10.8 kg/m³).

145 Fish were fed commercial pellets eight times per day at the beginning of exogenous feeding
146 (March) with a gradual decrease to reach one meal per day by November. We calculated rations so
147 that the food supplied was overestimated (commercial charts were designed for rainbow trout) and
148 to avoid having an excess of unsalted food that would decrease water quality. We stopped
149 supplying pellets when fish stopped eating, thus satiety was ensured. Fish were hand fed each
150 morning except in winter (December to end of March), when they were fed twice a week. Fish
151 were weighed at regular intervals and fin markings were verified. With this information, feeding
152 rations were modified and care was taken not to exceed a rearing load greater than 30 kg m⁻³. Fish
153 numbers were reduced when this was the case, with no attempt to keep the highest-performing fish,
154 and family pools in the different tanks were randomly modified except to avoid having similar
155 family fin marks in the same tank.

156

Commenté [MV2]: Combien de temps après ? il manque une échelle de temps dans cette phrase et la suivante

Mis en forme : Surlignage

Mis en forme : Surlignage

Commenté [MV3]: Porte à confusion, car on se demande quel est le cas particulier. J'aurais enlevé, ou alors précisé

Mis en forme : Surlignage

Mis en forme : Surlignage

Commenté [MV4]: Préciser le calcul (quel pourcentage de biomasse ? 2% ?

157 **2.3. Sampling**

158 We used the progeny of 13 families from the selection line and 16 families from the control
159 lines, both from the F5 generation (n = 4471 individuals: 2078 selected and 2393 control).
160 Individuals from the four families with the largest mean weight (high-performing families) and the
161 four families with the lowest mean weight (low-performing families) were sampled in both the
162 control and selected lines (Table 1, Fig. 1). Fish from each family were weighed (± 0.1 g) and
163 measured (± 0.1 cm) in July at the age of 7 months, in November at 11 months, and in June at 18
164 months (1+) (Suppl. Fig. 1); the phenotypes at 18 months of age were used to rank individuals and
165 families for this study. Fish were not fed for 24 h and then were anaesthetized (3-aminobenzoic
166 acid ethyl ester, 0.16 g L⁻¹) prior to measurements (length and weight). Fulton's condition factor
167 (K) (Fulton, 1904) was calculated as

168
$$K = (W L^{-3}) * 100$$

169 where W is the weight in g and L is the fork length in cm.

170 For each family (Table 1), the eight heaviest (fast-growing individuals) and the eight
171 lightest (slow-growing individuals) juveniles were sacrificed by severing the spinal cord and used
172 for further molecular analyses (Table 2, Fig. 1). The pituitary gland, brain, liver, and white muscle
173 were immediately removed and placed in sterile tubes, frozen in liquid nitrogen, and stored at -80°C
174 pending analyses.

175

176 **2.4. Total RNA and cDNA synthesis**

177 For each fish, liver and brain total RNA were extracted from 30 mg wet weight of tissue.
178 For pituitary RNA extraction, a pool of eight individuals from a same group was used because of

179 the gland's small size (Fig. 1), which prevented analysis at the individual level. RNA extractions
180 were performed using the RNeasy Plus Universal Mini Kit (liver, pituitary, and brain; Qiagen, Inc.,
181 Mississauga, ON, Canada) and RNeasy Fibrous Tissue Kit (muscle; Qiagen, Inc., Mississauga,
182 ON, Canada). Extracted RNA was diluted to a final concentration of 200 ng μL^{-1} . RNA purity,
183 quality, and concentration were measured by SYBRSafe DNA Gel Stain 2% agarose gel
184 electrophoresis (Alpha Imager HP System, Alpha-Innotech, Alpha Software, Invitrogen, Inc., CA,
185 USA) with an absorbance ratio of 260/280 (NanoVue Plus spectrophotometer, GE Healthcare,
186 Pittsburgh, PA, USA). Reverse transcription of mRNA into complementary DNA (cDNA) was
187 performed in duplicate for each sample and then pooled using the Quantitect Reverse Transcription
188 Kit (Qiagen, Inc., Mississauga, ON, Canada). cDNA was diluted to a final concentration of 200 ng
189 μL^{-1} , separated into aliquots, and kept frozen at -20°C until further analysis. cDNA integrity and
190 concentrations (1.8–2.0) were verified using a NanoVue Plus spectrophotometer. The efficiency of
191 reverse transcription was verified by quantitative polymerase chain reaction (qPCR) using serial
192 dilutions of a representative pool of cDNA samples collected from different sampling sites and
193 compared to the ideal slope of -3.3.

194

195 **2.5. Primer design for target genes**

196 To evaluate the impact of selection on the growth regulation pathway, the expressions of
197 the genes present in different tissues were quantified in each sampled fish (except for the pituitary
198 gland for which we used family pools). These different tissues included brain (target genes *npv*,
199 *lep-r*), pituitary (target genes *gh*, *ghr-1*, *igf-1r*, *grl*, *srifr*), liver (target genes *igf-1*, *ghr-1*), and
200 muscle (target genes *igf1r*, *ghr-1*, *mhc*, *myog*).

201 The first step was to obtain the DNA sequences for *S. fontinalis* since sequences were not
202 available for this species. We designed primers from Arctic charr *Salvelinus alpinus* and rainbow
203 trout *Oncorhynchus mykiss* sequences (Table 3) to perform PCR and amplify products of interest
204 in *S. fontinalis*. PCR was performed in 25 μ L reactions containing 12.5 μ L of AmpliTaq Gold 360
205 (Applied Biosystems), 0.5 μ L of 360 GC enhancer (Applied Biosystems), 2.5 μ L of cDNA, 1.25
206 μ L each of forward and reverse primer (20 mM), and 7 μ L of nuclease-free H₂O. Reactions were
207 amplified under a thermal profile of 95°C for 10 min, 40 cycles at 95°C for 30 s, 60°C for 30 s,
208 and 72°C for 1 min and 20 s, followed by 7 min at 72°C. PCR products were then tested by gel
209 electrophoresis on 2% agarose gels. The amplified PCR products were purified using the QIAquick
210 PCR purification kit (Qiagen) and in forward and reverse sequences using the BigDye Terminator
211 v3.1 Cycle Sequencing kit (Applied Biosystems) with the ABI PRISM 3130 Genetic Analyzer
212 (Applied Biosystems). For each gene, the sequence obtained was compared with the sequence used
213 for primer design with the BLAST® software (Altschul *et al.*, 1990). Sequence lengths and
214 percentages of similarity to the reference sequences are presented in Table 3.

215

216 **2.6. Measurement of gene expression by qPCR**

217 Gene expression was measured by qPCR using the TaqMan technology, which involved
218 designing primers and probes specific to brook charr based on the gene sequences obtained in the
219 step described above. For pituitary analyses, IDT PrimeTime probes (Table 4) were designed using
220 the PrimerQuest tool (Integrated DNA Technologies, Coralville, IA, USA). For brain, liver, and
221 muscle, TaqMan probes (Table 5) were designed using the Primer Express software version 3.0
222 (Applied Biosystems). For all samples, qPCR gene expression was performed in triplicate using a
223 QuantStudio 3 Real Time PCR System (Applied Biosystems). Each reaction consisted of 2 μ L of

224 diluted cDNA, 5 μ L of TaqMan Fast Advanced Mix, 0.5 μ L of Custom TaqMan Gene Expression
225 Assay, and 2.5 μ L of sterile water, for a total volume of 10 μ L.

226 The thermal cycling of qPCR was done in two steps: (1) 2 min at 50°C for optimal
227 AmpErase uracil-N-glycosylase activity followed by 20 s at 95°C to activate DNA polymerase,
228 and (2) 45 denaturation cycles for 1 s at 95°C and annealing / extension for 20 s at 60°C. Cycle
229 thresholds (CT) were obtained with the QuantStudio Design Analysis software (ThermoFisher
230 Connect). The relative quantification of gene expression was calculated using the $2^{-\Delta\Delta C_T}$ method of
231 Livak and Schmittgen (2001), with C_T being a threshold cycle:

232

$$233 \quad 2^{-\Delta\Delta C_T} = 2^{-(\Delta C_{T_e} - \Delta C_{T_c})}$$

234

235 where $C_{T_e} = C_T$ of the candidate gene - C_T of the reference genes for sample x, and $C_{T_c} = C_T$ of the
236 target gene - C_T of the reference genes for the calibrator.

237 In this study, the calibrator was the CLS group (control line + low-performing families +
238 slow-growing phenotype). The stability of reference gene expressions between groups was verified
239 with Expression Suite version 1.0, where the score was calculated according to Vandesompele *et*
240 *al.* (2002). The reference genes were *18s*, *β -actin*, and *efl α* , and the best score combination
241 obtained with the QuantStudio Analysis software was kept for each tissue. For accurate averaging
242 of the control genes, we used the geometric mean instead of the arithmetic mean because the former
243 better controls for possible outlier values and abundance differences between the different genes
244 (Vandesompele *et al.*, 2002).

245

246 **2.7. Statistical analyses**

247 **2.7.1. Weight, length, and condition**

248 One-way ANOVAs ($\alpha < 0.05$) were used to compare family lengths, weights, and Fulton
249 condition factors within lines. This allowed us to compare growth performance among families and
250 to select those families used for gene expression as well as the slow- and fast- growing individuals
251 within each family. Data normality was verified using the Kolmogorov-Smirnov test and
252 homoscedasticity was tested using the Levene test (Statistica, version 6.1.478, Statsoft). When
253 ANOVA revealed significant differences between groups, we used post-hoc HSD Tukey tests if
254 homoscedasticity was verified and multiple-range Games-Howell tests if there was
255 heteroscedasticity.

256 **2.7.2. Gene expression**

257 Data outliers for *grl* (n = 1), *srifr* (n = 2), pituitary *ghr-1* (n = 1), white muscle *myog* (n =
258 1), and weight (n = 1) were removed before running analyses. For gene expression **in all tissues**
259 **except pituitary, n was the number of individuals (three individuals per family; Fig. 1A).** In the
260 pituitary, the statistical n was the number of families per line and not the number of individuals.
261 Because we had to pool individuals to obtain enough biological material **(eight individuals per**
262 **family; Fig. 1B),** no family effect was assessed. Prior to analyses, the following data
263 transformations were applied to achieved normality: log transformations for pituitary *grl* and *srifr*
264 and liver *ghr-1*; Box-Cox transformations for pituitary *igf-1r* and *ghr-1*, brain *npv* and *lepr*, liver
265 *igf-1*, and white muscle *igf-1r*, *ghr-1*, and *mhc*. For each selected gene in the pituitary, separate
266 linear mixed models (LMM) or linear models (LM) were built that related gene expression to
267 length, condition (Fulton index), and line (control or selected) (R version 4.0.5 package lme4).
268 Family identity was first included in all non-pituitary models as a random effect. Models were

269 simplified by a backward elimination procedure, where the least significant term (based on P -
270 value) was sequentially removed until all remaining variables were significant (*i.e.*, $P < 0.05$,
271 confirmed by a Likelihood Ratio Test). Body weight was not included in the models due to its
272 strong positive correlation with length ($R = 0.937$). Marginal and conditional R-squared values
273 were obtained using the rsquared function from the piecewiseSEM R package (Lefcheck, 2016).

274 ~~Gene expressions~~ was/were compared between lines, family and individuals using two-way
275 nested ANOVAs (factors: line and family; individual performance nested in “family”). Normality
276 and homoscedasticity were tested and a posteriori tests were run as previously described. Finally,
277 the relationships between growth variables (weight, length, and condition) were analyzed using
278 simple linear regressions.

279

280

281 3. RESULTS

282 3.1. Weight, length, and condition differences between control and selected lines

283 On average, fish from the selected line were 37.21% heavier than fish from the control line
284 ($11.95 \text{ g} \pm 4.57$ vs $8.71 \text{ g} \pm 3.36$; $F_{(1,4470)} = 740.42$; $p < 0.001$; Fig. 2A and 2B). Their length (10.76
285 $\text{cm} \pm 1.38$) was also 11.54% greater than the control line ($9.65 \text{ cm} \pm 1.26$; $F_{(1,4470)} = 793.96$;
286 $p < 0.001$; Fig. 2C and 2D). However, the condition factor of control line fish was significantly
287 (albeit only slightly) higher than that of the selected line (0.93 ± 0.14 vs 0.92 ± 0.12 ; $F_{(1,4470)} =$
288 6.98 ; $p = 0.0083$; Fig. 2E and 2F).

289

3.2. Weight, length, and condition differences among families [within lines](#)

Even though the selection process lasted five generations, family effects were still very present in both the selected and control lines. In the selected line, weights of the best- and the least-performing families differed by 49.42% ($F_{(12,2077)} = 35.79$; $p < 0.001$) while they differed by 14.82% in the control line ($F_{(15,2392)} = 32.76$; $p < 0.001$).

It is noteworthy that the family with the lowest weight in the selected line was significantly different from the rest of the selected families, from those control line families that had average and low performance (Fig. 2A and 2B).

This same family effect was also observed for length in the selected line: the largest family was significantly bigger—by 7.63%—than the smallest family ($F_{(12,2077)} = 24.96$; $p < 0.001$), and this was even more evident in the control line, where the difference was 14.62% ($F_{(15,2392)} = 35.34$; $p < 0.001$; Fig. 2C and 2D).

Weight and length were significantly positively correlated in the selected line ($F_{(1,2076)} = 12814.51$; $p < 0.001$; Length = $0.28 * \text{Weight} + 7.32$; $R = 0.927$) as well as in the control line ($F_{(1,2392)} = 16228.92$; $p < 0.001$; Length = $0.3493 * \text{Weight} + 6.60$; $R = 0.933$). However, significant albeit very slight correlations were found between condition factor and weight ($F_{(1,4469)} = 15.03$; $p < 0.001$, $R = 0.057$) and between condition and length ($F_{(1,4469)} = 238.36$; $p < 0.001$, $R = 0.225$).

3.3. Gene expression

3.3.1. Selection and family performance [within lines](#)-based effects on gene expression

In the brain, *npv* and *lepr* gene expressions were not different between lines (Table 6), but *lepr* expression was significantly higher in low-performing families ($F_{(1,44)} = 6.85$; $p = 0.012$); no

312 family effect was found in *npv* (Table 6). The expression of these two genes was not linked to
313 length or condition (Table 6).

314 Pituitary *grl*, *srifr*, *gh*, and *ghr-1* gene expressions were not different between lines (Table
315 6). The expression of these genes was not significantly linked to length or condition (Table 6).
316 While pituitary *igf-1r* gene expression was not different between lines ($p = 0.80$), it was
317 significantly positively linked to length (Table 6).

318 In liver, *ghr-1* and *igf-1* gene expressions were significantly higher in the selected line
319 compared to the control line (Table 6, Fig. 3A and 3C), but no family effect was found (Table 6).
320 Expression of the *ghr-1* gene was negatively impacted by condition but not by length (Table 6, Fig.
321 3B). On the contrary, *igf-1* gene expression was positively associated with length but not with
322 condition (Table 6, Fig. 3D).

323 In white muscle, the relative expressions of *mhc*, *ghr-1*, and *myog* were not significantly
324 different between lines (Table 6), while *igf-1r* gene expression was significantly higher in the
325 selected line (Table 6, Fig. 3E). *Mhc*, *ghr-1*, and *igf-1r* gene expressions were not different among
326 families, but we found a significant family effect in *myog* gene expression (Table 6). Nevertheless,
327 the complementary ANOVA analysis did not show significant differences among families with
328 low and high performance ($F = 2.8$, $p = 0.09$). Relative expressions of *mhc*, *myog*, and *igf-1r* were
329 not impacted by length (Table 6), but *ghr-1* expression significantly increased with length (Table
330 6, Fig. 3F). None of the genes quantified in white muscle (*mhc*, *ghr-1*, *myog*, *igf-1r*) were linked
331 to condition (Table 6).

332

333 **3.3.2. Individual performance within families-based effects**

334 Phenotypes were compared between low- and high-performing families; selected and
335 control families were combined since no significant line effect was found (Suppl. Table 1). Brain
336 *npv* and *lepr* gene expressions were higher in slow-growing individuals than in fast-growing
337 individuals from both low- and high-performing families (respectively $F_{(1,91)} = 5.26$; $p = 0.02$; $F_{(1,91)}$
338 $= 6.70$; $p < 0.001$) (Fig. 4A and 4B).

339 In liver, the relative expression of *ghr-1* showed no line \times family interactions ($F_{(1,91)} = 0.20$;
340 $p = 0.64$), but it differed according to both family and individual performances. The *ghr-1*
341 expression was higher in fast-growing juveniles from high-performing families ($F_{(2,91)} = 24.31$;
342 $p < 0.001$), but no difference was observed in low-performing families ($F_{(2,91)} = 1.37$; $p = 0.24$)
343 (Fig. 4C).

344 The same nested effect was observed for liver *igf-1*, with no line \times family interactions
345 ($F_{(1,91)} = 0.87$; $p = 0.35$), a higher expression in the fast-growing juveniles in high-performing
346 families ($F_{(2,91)} = 15.75$; $p < 0.001$), and no differences in low-performing families ($F_{(2,91)} = 2.17$;
347 $p = 0.14$) (Fig. 4D).

348 In muscle, *ghr-1* expression showed no line \times family interactions ($F_{(1,91)} = 0.33$; $p = 0.56$).
349 A nested effect was observed, with higher expression in the fast-growing juveniles from families
350 with both low and high performance ($F_{(1,91)} = 4.23$; $p = 0.01$) (Fig. 4E). No nested effect or
351 interactions were observed in the relative expression of *igf-1r* ($F_{(1,91)} = 2.6$; $p = 0.07$), *mhc* ($F_{(1,91)}$
352 $= 2.42$; $p = 0.09$), or *myog* ($F_{(1,91)} = 0.01$; $p = 0.98$).

353

354 4. DISCUSSION

355 In this study, we tested the hypothesis that selection enhanced the differential expression of genes
356 involved in the *gh/igf-1* axis, in appetite control, and in muscle growth between fish with slow- and
357 fast-growing phenotypes and between families with different growth performance. **When**
358 **comparing the two selected and control lines, we were looking for selection on endocrine traits,**
359 **and when comparing family and individual traits within lines, we looked for differences resulting**
360 **from both physiological and endocrine traits.** As expected, fish from the selected line were heavier
361 and larger than those from the control line. However, our results did not support our main
362 hypothesis—that selection enhanced differential expressions of the examined target genes involved
363 in the GH/IGF-1 axis between slow- and fast-growing phenotypes. Indeed, growth performance in
364 the selected line was associated with a higher relative expression of liver *igf-1* and muscle *igf1-r*,
365 but not with genes controlling appetite or muscle growth. However, some genes related to appetite
366 control or muscle growth were linked to family performance and individual phenotypes, raising
367 interesting questions about factors underlying non-selection-based phenotypic variations.

368

369 4.1. Selection-based effects on gene expression

370 Our results showed an upregulation of the *gh/igf-1* axis, starting with pituitary *igf-1r* and
371 followed by liver *ghr-1*, liver *igf-1*, muscle *ghr-1*, and muscle *igf-1r* in the selected line, clearly
372 indicating an effect of selection on this axis (Fig. 5). The only gene we examined on this axis that
373 was not upregulated in the selected line was pituitary *gh*. While this may be explained by the limited
374 statistical power for the analysis of pituitary gene expression considering that samples were pooled

375 in the analysis, we nevertheless consider this unlikely because differences in pituitary *igf-1r* were
376 detected.

377 In other fast-growing salmonids that had followed a simple selection process based only on
378 mass, the upregulation of liver *igf-1* and muscle *igf-1r* combined with positive growth correlation
379 is well known (Fleming *et al.*, 2002; Devlin *et al.*, 2009; Tymchuk *et al.*, 2009). It appears that
380 selection based on growth and the absence of early sexual maturation in brook charr also enhanced
381 weight gain via upregulation of the *gh/igf-1* axis. It is noteworthy that the upregulation of liver *ghr-*
382 *1* promotes the synthesis of *igf-1* in the liver. Indeed, mRNA levels of *igf-1*, *igf-1r*, and *gh* had
383 already been identified as genes of interest for promoting growth in the same strain of *S. fontinalis*
384 (Sauvage *et al.*, 2012). Such upregulation was shown to enhance lipid catabolism to obtain energy
385 for growth in Atlantic salmon *Salmo salar* (Hevrøy *et al.*, 2015), which could explain the improved
386 condition factor in the selected line. Even though the selection process did not modify the
387 expression of pituitary *srifr*, this does not mean that a reduction in the production of SRIF, an
388 inhibitor of growth hormone synthesis, could not be occurring. It should be noted that the effects
389 of SRIF on *gh* expression are limited and conflicting (Wang *et al.*, 2016).

390 We showed that 1+ *S. fontinalis* juveniles reared under the same conditions, including
391 temperature and food rations, displayed a differential modulation of the *gh/igf-1* axis, which may
392 have been enhanced by the selection process itself and not modulated by the influence of rearing
393 variables such as stress (Meier *et al.*, 2009; Nakano *et al.*, 2013), feeding, or fasting (Chauvigné *et*
394 *al.*, 2003; Fukada *et al.*, 2004; Norbeck *et al.*, 2007; Bower *et al.*, 2008), diet composition (Gomez-
395 Requeni *et al.*, 2005; Hack *et al.*, 2018), or temperature (Hevrøy *et al.*, 2013), as previously
396 documented in other salmonids. It is important to note that temperature is one of the most dominant
397 factors influencing some key biological functions in fish—including food ingestion—that decrease

398 at higher or lower temperatures (Assan et al., 2021). Winter temperature did not slow growth
399 (Suppl. Fig. 2), and even though specific family growth rate (SGR) was generally lower from 7 to
400 11 months of age (July to November) than from 11 to 18 months (November to June), rankings
401 remained roughly the same and confirmed that phenotype differences were consistent between lines
402 through time and representative of the phenotypes measured in 18-month-old fish.

403 We had expected to find a difference in appetite control (*npv* in particular), but found no
404 difference in relative gene expression between the two lines. Yet, it was previously reported that
405 selection had an impact on food intake in Atlantic salmon, promoting faster growth and also
406 improving the efficient utilization of proteins and energy (Gjedrem and Baranski, 2009). Again,
407 we did not find any differences in the relative expression of muscle *mhc* and *myog* between lines.
408 However, the absence of gene expression may not necessarily mean an absence of protein activity.
409 It should also be noted that these two genes are only involved in the final stages of myocyte
410 development (differentiation and maturation) (Evans *et al.*, 2014). Differences could have been
411 present in the expression of muscle genes involved in the first stage of activation, such as nuclear
412 antigen in proliferating cells (*pcna*) or in cell proliferation with the expression of different
413 transcription factors, such as *Sox8*, *Myf5*, *MyoD2*, and *Pax7* (Vélez *et al.*, 2017), which activate
414 intracellular transduction cascades via *igf-1* receptors (Dupont and LeRoith, 2001; Hack *et al.*,
415 2018).

416

417 **4.2. Family performance within lines-based effects on gene expression**

418 Contrary to what we observed between selected and control lines, family performance was
419 related to the expressions of both *lepr* and *myog* for both the selection and control lines. Relative
420 *lepr* expression was upregulated in low-performing families, suggesting suppressed food intake

421 and increased metabolism, resulting in increased energy expenditure and weight loss (Klok *et al.*,
422 2007; Volkoff, 2016; Blanco and Soengas, 2021). In contrast, high-performing families did not
423 show any difference in *lepr* expression but rather an upregulation of muscle *ghr* that could enhance
424 growth; this has been widely demonstrated in several teleosts (Picha *et al.*, 2008; Hevrøy *et al.*,
425 2013, 2015; Vélez *et al.*, 2017).

426

427 **4.3. Individual performance within families-based effects**

428 In slow-growing juveniles, differences in appetite regulation may be due to *lepr*
429 upregulation (Fig. 6) since the binding of leptin to *lepr* activates the Jak/STAT intracellular
430 signaling pathways, which decreases food intake by down regulating other neuropeptides such as
431 NPY (Blanco and Soengas, 2021; Volkoff *et al.*, 2003). Conversely, the expression of *npv* was also
432 upregulated in slow-growing fish, although the exact mechanism of action triggered after leptin
433 binding to *lepr* is unknown in teleost fish (Blanco and Soengas, 2021). The relative weight of these
434 two mechanisms on appetite regulation cannot be assessed without food intake experiments, which
435 should certainly be a focus in further studies. Also, we cannot refute the possibility of differences
436 in appetite or food consumption that may have occurred among individuals or families throughout
437 the experiment. Nevertheless, we are confident that maintaining an equal load in each rearing tank,
438 feeding to satiation, and grouping the families differently at regular intervals helped to maintain
439 dominance and family hierarchy at the lowest possible levels. **Despite these precautions, we cannot
440 rule out that size variation could partly be the result of aggression, with some fish not feeding
441 maximally, which would result in reduced growth rates.**

442 We found no indication of differences related to muscle growth regulation. In future studies,
443 it would be relevant to look for differences in the PI3/Akt/TOR pathway (the central mediator in

444 the nutrient sensing protein pathway and precursor of many myogenic factors), which is only
445 activated by feeding. Upregulation of this pathway was recently reported in fast-growing *O. mykiss*
446 (Cleveland *et al.*, 2020). Modifications in the trajectory of growth antagonist genes (e.g., precursors
447 to the alpha subunits of Meprin A) (Valente *et al.*, 2013; Evans *et al.*, 2014), which were strongly
448 expressed in the Laval control line in brook charr (Sauvage *et al.*, 2010), should also be assessed.

449

450 **4.4. Impact of selection on general growth across generations**

451 In the first generations following initiation of the selective breeding programs with wild
452 breeders brought into captivity, Bastien *et al.* (2011) found that mean weight in the selected line
453 increased by 23.1% after the first generation, by 32.1% after the second, and by 4% after the third.
454 In our study, the combined selection showed that fish from the fifth generation of the selected line
455 showed a weight gain of 37.2% compared to those from the control line. In other salmonids, it is
456 known that genetic improvements produce permanent gains (Gjedrem and Baranski, 2009). Our
457 results are consistent with those of previous studies, such as Kause *et al.* (2005), who showed that
458 combined selection improved *O. mykiss* growth by 7% per generation over two generations, and
459 Gjerde and Korsvoll (1999) reported that Atlantic salmon after six generations showed
460 improvements in growth rate of 83.9% overall (14% per generation) and a 12.5% reduction in the
461 frequency of early sexual maturity.

462 Surprisingly, the condition factor in brook charr juveniles was lower in the selected line
463 than in the control line due to variable gains in weight and length in the selected line. This could
464 be explained by different regulations in the mechanisms related to energy reserves, as has been
465 mentioned for *igf-1* and lipid catabolism. A strongly significant positive correlation between
466 condition factor and total lipid content in Atlantic salmon suggests that condition factor can be used

467 to indicate the state of energy reserves rather than as an indicator of growth (Herbinger and Friars,
468 1991; Sutton *et al.*, 2000).

469

470 5. CONCLUSIONS

471 Selection for the absence of early maturation combined with selection for high growth rate resulted
472 in an upregulation of the *gh/igf-1* axis with no effect on the expression of genes related to appetite
473 control or muscle growth. In contrast, phenotype differences in both the selected and control lines
474 within families resulted in different expressions of genes related to appetite regulation. Slow-
475 growing fish were characterized by an upregulation of brain *lepr* and a downregulation of the
476 *gh/igf-1* axis. Overall, our results show that *lepr* could be used as a physiological indicator of
477 growth related to phenotypic variation and family performance. Liver *igf-1* as well as muscle *ghr*
478 and *igf-1r* gene expressions could be considered as indicators of good growth among brook charr
479 lines. The role of the receptors, which can only be studied with the transcriptomic approach, should
480 be included in future studies because of their importance in the growth regulation pathway. Further
481 research is needed to investigate which genes involved in muscle growth could be stimulated
482 through *gh/igf-1* axis upregulation. By identifying the molecular mechanisms by which *gh/igf-1*
483 signaling is modulated at the endocrine level (paracrine and autocrine), we should be able to better
484 understand growth patterns that optimize growth strategies in commercial fish production. Finally,
485 large weight and length variations still exist in the selected line, indicating that the full genetic
486 selection potential had not been reached after five generations.

487

488 **6. ACKNOWLEDGEMENTS**

489 We are grateful to all colleagues who provided technical and scientific assistance in the
490 laboratory. Special thanks to Renée Gagné (Institut des sciences de la mer de Rimouski) who
491 contributed to laboratory training, and Geneviève Parent, Éric Parent, and Grégoire Cortial
492 (Fisheries and Oceans Canada), who helped with sequencing. We also thank the journal editor
493 M.A. Sheridan and two anonymous reviewers for their constructive input on a previous version of
494 this manuscript. This research was supported by the Conseil de Recherche en Sciences Naturelles
495 et en Génie du Canada (CRSNG, subvention à la découverte N° RGPIN-2019-05739) and by the
496 *Ressources Aquatiques Québec* research network.

497

498

499 **7. REFERENCES**

- 500 Ahammad, A.K.S., Asaduzzaman, M., Asakawa, S., Watabe, S., and Kinoshita, S., 2015.
501 Regulation of gene expression mediating indeterminate muscle growth in teleosts. *Mech. Dev.*
502 137, 53–65.
- 503 Aldegunde, M., and Mancebo, M., 2006. Effects of neuropeptide Y on food intake and brain
504 biogenic amines in the rainbow trout (*Oncorhynchus mykiss*). *Peptides*. 27, 719–727.
- 505 Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J., 1990. Basic local alignment
506 search tool. *J. Mol. Biol.* 215, 403–410.
- 507 Anderson, J.H., Faulds, P.L., Atlas, W.I., Pess, G.R., and Quinn, T.P., 2010. Selection on breeding
508 date and body size in colonizing coho salmon, *Oncorhynchus kisutch*. *Mol. Ecol.* 19, 2562–
509 2573.
- 510 Assan D., Huang Y., Mustapha, U.F., Addah, M.N., Li, G., and Chen, H. 2021. Fish feed intake,
511 feeding behavior, and the physiological response of apelin to fasting and refeeding. *Front.*
512 *Endocrinol.* 12, 1–12.
- 513 Bastien, A., Perry, G.M.L., Savaria, J., Bernatchez, L., and Audet, C., 2011. Genetic gain for
514 growth and delayed sexual maturation using a feral strain of anadromous brook trout. *N. Am.*

515 J. Aquac. 73, 24–33.

516 Björnsson, B.T., 1997. The biology of salmon growth hormone: from daylight to dominance. Fish
517 Physiol. Biochem. 17, 9–24.

518 Blanco, A.M., and Soengas, J.L., 2021. Leptin signalling in teleost fish with emphasis in food
519 intake regulation. Mol. Cell. Endocrinol. 526, 111209.

520 Bower, N.I., Li, X., Taylor, R., and Johnston, I.A., 2008. Switching to fast growth: the insulin-like
521 growth factor (IGF) system in skeletal muscle of Atlantic salmon. J. Exp. Biol. 211, 3859–
522 3870.

523 Breton, B., Mikolajczyk, T., Danger, J., Gonnet, F., Saint-Pierre, S., and Vaudry, H., 1989.
524 Neuropeptide Y (NPY) modulates in vitro gonadotropin in release from rainbow trout
525 pituitary glands. Fish Physiol. Biochem. 7, 77–83.

526 Cerdá-Reverter, J.M., and Larhammar, D., 2000. Neuropeptide Y family of peptides: Structure,
527 anatomical expression, function, and molecular evolution. Biochem. Cell Biol. 78, 371–392.

528 Chauvigné, F., Gabillard, J.C., Weil, C., and Rescan, P.Y., 2003. Effect of refeeding on IGFI,
529 IGFI, IGF receptors, FGF2, FGF6, and myostatin mRNA expression in rainbow trout
530 myotomal muscle. Gen. Comp. Endocrinol. 132, 209–215.

531 Cleveland, B.M., Gao, G., and Leeds, T.D., 2020. Transcriptomic response to selective breeding
532 for fast growth in rainbow trout (*Oncorhynchus mykiss*). Mar. Biotechnol. 22, 539–550.

533 Clutton-Brock, T.H., 1988. Reproductive success: studies of individual variation in contrasting
534 breeding systems. University of Chicago Press, Chicago.

535 Crespel, A., Bernatchez, L., Garant, D., and Audet, C., 2013. Genetically based population
536 divergence in overwintering energy mobilization in brook charr (*Salvelinus fontinalis*).
537 Genetica. 141, 51–64.

538 Dar, S.A., Srivastava, P.P., Varghese, T., Gupta, S., Gireesh-Babu, P., and Krishna, G., 2018.
539 Effects of starvation and refeeding on expression of ghrelin and leptin gene with variations in
540 metabolic parameters in *Labeo rohita* fingerlings. Aquaculture. 484, 219–227.

541 Devlin, R.H., Sakhrani, D., Tymchuk, W.E., Rise, M.L., and Goh, B., 2009. Domestication and
542 growth hormone transgenesis cause similar changes in gene expression in coho salmon
543 (*Oncorhynchus kisutch*). Proc. Natl. Acad. Sci. USA. 106, 3047–3052.

544 Duan, C., Ren, H., and Gao, S., 2010. Insulin-like growth factors (IGFs), IGF receptors, and IGF-
545 binding proteins: Roles in skeletal muscle growth and differentiation. Gen. Comp. Endocrinol.

546 167, 344–351.

547 Dupont, J., and LeRoith, D., 2001. Insulin and insulin-like growth factor I receptors: similarities
548 and differences in signal transduction. *Horm. Res.* 55, 22–26.

549 Evans, D.H., Claiborne, J.B., and Currie, S., 2014. *The physiology of fishes*. CRC Press, Boca
550 Raton, FL. 480 p.

551 Fleming, I.A., Agustsson, T., Finstad, B., Johnsson, J.I., and Björnsson, B.T., 2002. Effects of
552 domestication on growth physiology and endocrinology of Atlantic salmon (*Salmo salar*).
553 *Can. J. Fish. Aquat. Sci.* 59, 1323–1330.

554 Fukada, H., Ozaki, Y., Pierce, A.L., Adachi, S., Yamauchi, K., Hara, A., Swanson, P., 2004.
555 Salmon growth hormone receptor: molecular cloning, ligand specificity, and response to
556 fasting. *Gen. Comp. Endocrinol.* 139, 61–71.

557 Fulton, T.W., 1904. The rate of growth of fishes. 22nd Ann. Rep. Fish. Board Scotl. 3, 141–241.

558 Gjedrem, T., 2005. *Selection and breeding programs in aquaculture*. Springer, Dordrecht. 364 p.

559 Gjedrem, T., and Baranski, M., 2009. *Selection breeding in aquaculture: An introduction*. Springer,
560 London. 848 p.

561 Gjerde, B., and Korsvoll, A., 1999. Realized selection differentials for growth rate and early sexual
562 maturity in Atlantic salmon. *Aquac. Eur.* 27, 73–74.

563 Gomez-Requeni, P., Calduch-Giner, J., Vega-Rubín de Celis, S., Médale, F., Kaushik, S.J., and
564 Pérez-Sánchez, J., 2005. Regulation of the somatotropic axis by dietary factors in rainbow
565 trout (*Oncorhynchus mykiss*). *Br. J. Nutr.* 94, 353–361.

566 Hack, N.L., Strobel, J.S., Journey, M.L., Beckman, B.R., and Lema, S.C., 2018. Response of the
567 insulin-like growth factor-1 (*Igf1*) system to nutritional status and growth rate variation in
568 olive rock fish (*Sebastes serranoides*). *Comp. Biochem. Physiol., Part A.* 224, 42–52.

569 Herbinger, C.M., and Friars, G.W., 1991. Correlation between condition factor and total lipid
570 content in Atlantic salmon, *Salmo salar* L. parr. *Aquacul. Fish. Manag.* 22, 527–529.

571 Hessen, D.O., Jeyasingh, P.D., Neiman, M., and Weider, L.J., 2010. Genome streamlining and the
572 elemental costs of growth. *Trends Ecol. Evol.* 25, 75–80.

573 Hevrøy, E.M., Hunskaar, C., de Gelder, S., Shimizu, M., Waagbø, R., Breck, O., and Takle, H.,
574 2013. GH-IGF system regulation of attenuated muscle growth and lipolysis in Atlantic salmon
575 reared at elevated sea temperatures. *J. Comp. Physiol. B: Biochem. System. Environ. Physiol.*
576 183, 243–259.

577 Hevrøy, E.M., Tipsmark, C.K., Remø, S.C., Hansen, T., Fukuda, M., Torgersen, T., Vikeså, V.,
578 2015. Role of the GH-IGF-1 system in Atlantic salmon and rainbow trout postsmolts at
579 elevated water temperature. *Comp. Biochem. Physiol. A : Mol. Integr. Physiol.* 188, 127–138.

580 Hoskins, L.J., and Volkoff, H., 2012. The comparative endocrinology of feeding in fish: Insights
581 and challenges. *Gen. Comp. Endocrinol.* 176, 327–335.

582 Kause, A., Ritola, O., Paananen, T., Wahloos, H., and Mäntysaari, E., 2005. Genetic trends in
583 growth, sexual maturity and skeletal deformations, and rate of inbreeding in a breeding
584 programme for rainbow trout (*Onchorhynchus mykiss*). *Aquaculture.* 247, 177–187.

585 Kawaguchi, K., Kaneko, N., Fukuda, M., Nakano, Y., Kimura, S., Hara, A., and Shimizu, M., 2013.
586 Responses of insulin-like growth factor (IGF)-I and two IGF-binding protein-1 subtypes to
587 fasting and re-feeding, and their relationships with individual growth rates in yearling masu
588 salmon (*Oncorhynchus masou*). *Comp. Biochem. Physiol. A.* 165, 191–198.

589 Klok, M.D., Jakobsdottir, S., and Drent, M.L. 2007. The role of leptin and ghrelin in the regulation
590 of food intake and body weight in humans: A review. *Obes. Rev.* 8, 21–34.

591 Lefcheck, J.S., 2016. PiecewiseSEM: Piecewise structural equation modeling in R for ecology,
592 evolution, and systematics. *Methods Ecol. Evol.* 7(5), 573–579.

593 Livak, K J., and Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time
594 quantitative PCR and the 2 ddCT. *Methods.* 25, 402–408.

595 Meier, K.M., Figueiredo, M.A., Kamimura, M.T., Laurino, J., Maggioni, R., Pinto, L.S.,
596 Dellagostin, O.A., 2009. Increased growth hormone (GH), growth hormone receptor (GHR),
597 and insulin-like growth factor I (IGF-I) gene transcription after hyperosmotic stress in the
598 Brazilian flounder *Paralichthys orbignyanus*. *Fish Physiol. Biochem.* 35, 501–509.

599 Nakano, T., Afonso, L.O.B., Beckman, B.R., Iwama, G.K., and Devlin, R.H., 2013. Acute
600 physiological stress down-regulates mRNA expressions of growth-related genes in coho
601 salmon. *PLoS ONE.* 8, 1–7.

602 Nelson, L.E., and Sheridan, M.A., 2005. Regulation of somatostatins and their receptors in fish.
603 *Gen. Comp. Endocrinol.* 142, 117–133.

604 Nilsson, J.A.N., 1990. Heritability estimates of growth-related traits in Arctic charr (*Salvelinus*
605 *alpinus*). *Aquaculture.* 84, 211–217.

606 Norbeck, L.A., Kittilson, J.V.D., and Sheridan, M.A., 2007. Resolving the growth-promoting and
607 metabolic effects of growth hormone: Differential regulation of GH – IGF-I system

608 components. Gen. Comp. Endocrinol. 151, 332–341.

609 Pankova, M.V., Kухlevsky, A.D., and Brykov, V.A., 2017. Fish growth hormone genes:
610 Divergence of coding sequences in salmonid fishes. Russ. J. Genet. 53, 221–232.

611 Perelló-Amorós, M., Vélez, E.J., Vela-Albesa, J., Sanchez-Moya, A., Riera-Heredia, N., Heden,
612 I. 2018. Ghrelin and its receptors in gilthead sea bream: nutritional regulation. Front.
613 Endocrinol. 9(339), 1–14.

614 Perry, G.M.L., Audet, C., Laplatte, B., and Bernatchez, L., 2004. Shifting patterns in genetic
615 control at the embryo-alevin boundary in brook charr. Evolution. 58, 2002–2012.

616 Perry, G.M.L., Audet, C., and Bernatchez, L., 2005. Maternal genetic effects on adaptive
617 divergence between anadromous and resident brook charr during early life history. J. Evol.
618 Biol. 18, 1348–1361.

619 Picha, M.E., Turano, M.J., Beckman, B.R., and Borski, R.J. 2008. Endocrine biomarkers of growth
620 and applications to aquaculture: A minireview of growth hormone, insulin-like growth factor
621 (IGF -I), and IGF-binding proteins as potential growth indicators in fish. N. Am. J. Aquac.
622 70, 196–211.

623 Rønnestad, I., Gomes, A.S., Murashita, K., Angotzi, R., Jönsson, E., Volkoff, H. 2017. Appetite-
624 controlling endocrine systems in teleosts. Front. Endocrinol. 8, 1–24.

625 Sauvage, C., Derome, N., Normandeau, E., St.-Cyr, J., Audet, C., and Bernatchez, L., 2010. Fast
626 transcriptional responses to domestication in the brook charr *Salvelinus fontinalis*. Genetics.
627 185, 105–112.

628 Sauvage, C., Vagner, M., Derome, N., Audet, C., and Bernatchez, L., 2012. Coding gene SNP
629 mapping reveals QTL linked to growth and stress response in brook charr (*Salvelinus*
630 *fontinalis*). G3-Genes Genom. Genet. 2, 707–720.

631 Sheridan, M.A., and Hagemeister, A.L., 2010. Somatostatin and somatostatin receptors in fish
632 growth. Gen. Comp. Endocrinol. 167, 360–365.

633 Skaara, T., and Regenstein, J.M., 1990. The structure and properties of myofibrillar proteins in
634 beef, poultry, and fish. J. Muscle Foods. 1, 269–291.

635 Sutton, S.G., Bult, T.P., and Haedrich, R.L., 2000. Relationships among fat weight, body weight,
636 water weight, and condition factors in wild Atlantic salmon parr. Trans. Am. Fish. Soc. 129,
637 527–538.

638 Tymchuk, W., Sakhriani, D., and Devlin, R., 2009. Domestication causes large-scale effects on

639 gene expression in rainbow trout: Analysis of muscle, liver and brain transcriptomes. *Gen.*
640 *Comp. Endocrinol.* 164, 175–183.

641 Valente, L.M.P., Moutou, K.A., Conceição, L.E.C., Engrola, S., Fernandes, J.M.O., and Johnston,
642 I.A. 2013. What determines growth potential and juvenile quality of farmed fish species? *Rev.*
643 *Aquac.* 5, 168–193.

644 Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., and Speleman,
645 F., 2002. Accurate normalization of real-time RT-PCR data by geometric averaging of
646 multiple internal control genes. *Genom. Biolo.* 3, 1–13.

647 Vélez, E.J., Lutfi, E., Azizi, S., Monserrat, N., Riera-Codina, M., Capilla, E., Navarro, I., 2016.
648 Contribution of in vitro myocytes studies to understanding fish muscle physiology. *Comp.*
649 *Biochem. Physiol. B: Biochem. Mol. Biol.* 199, 67–73.

650 Vélez, E.J., Lutfi, E., Azizi, S., Perelló, M., Salmerón, C., Riera-Codina, M., Ibarz, A., 2017.
651 Understanding fish muscle growth regulation to optimize aquaculture production.
652 *Aquaculture.* 467, 28–40.

653 Very, N.M., and Sheridan, M.A., 2007. Somatostatin inhibits insulin-like growth factor-I receptor
654 expression in the gill of a teleost fish (*Oncorhynchus mykiss*). *FEBS Letters.* 581, 4773–4777.

655 Volkoff, H., 2016. The neuroendocrine regulation of food intake in fish: A review of current
656 knowledge. *Front. Neurosci.* 10, 1–31.

657 Volkoff, H., Eykelbosh, A.J., Peter, R.E., 2003. Role of leptin in the control of feeding of goldfish
658 *Carassius auratus*: interactions with cholecystokinin, neuropeptide Y and orexin A, and
659 modulation by fasting. *Brain Res.* 972, 90–109.

660 Volkoff, H., Hoskins, L.J., and Tuziak, S.M., 2010. Influence of intrinsic signals and environmental
661 cues on the endocrine control of feeding in fish: Potential application in aquaculture. *Gen.*
662 *Comp. Endocrinol.* 167, 352–359.

663 Wang, B., Jia, J., Yang, G., Qin, J., Zhang, C., Zhang, Q., Sun, C., 2016. In vitro effects of
664 somatostatin on the growth hormone-insulin-like growth factor axis in orange-spotted grouper
665 (*Epinephelus coioides*). *Gen. Comp. Endocrinol.* 237, 1–9.

666 Wood, A.W., Duan, C., and Bern, H.A. 2005. Insulin-like growth factor signaling in fish. *Int. Rev.*
667 *Cytol.* 243, 215–285.

668 Zhang, G., Chu, W., Hu, S., Meng, T., Pan, L., Zhou, R., Liu, Z., 2011. Identification and analysis
669 of muscle-related protein isoforms expressed in the white muscle of the Mandarin fish

670 (*Siniperca chuatsi*). J. Mar. Biotechnol. 13, 151–162.

671

672 **Table 1.** Growth characteristics of *Salvelinus fontinalis* 1+ families used in the study. Groups were
 673 formed according to line (C= control; S= selected) and family performance (L= low; H= high).

Group	Family	n	Weight (g)	Length (cm)	Condition (K)
CL	C19	142	6.14 ± 1.82	8.82 ± 0.79	0.87 ± 0.13
	C16	119	7.19 ± 2.23	9.34 ± 0.99	0.86 ± 0.14
	C1	164	7.21 ± 3.63	8.92 ± 1.31	0.95 ± 0.23
	C18	135	7.43 ± 2.76	9.07 ± 1.10	0.96 ± 0.18
CH	C22	99	9.59 ± 2.45	10.02 ± 0.91	0.93 ± 0.10
	C5	199	10.50 ± 3.42	10.36 ± 1.17	0.91 ± 0.12
	C17	156	10.77 ± 3.66	10.54 ± 1.27	0.89 ± 0.11
	C10	179	11.20 ± 4.00	10.50 ± 1.30	0.93 ± 0.11
SL	S1	147	8.14 ± 2.49	9.62 ± 1.07	0.89 ± 0.08
	S19	164	10.25 ± 3.94	10.21 ± 1.35	0.92 ± 0.17
	S5	157	10.59 ± 3.63	10.55 ± 1.25	0.87 ± 0.10
	S3	178	10.98 ± 4.35	10.25 ± 1.49	0.98 ± 0.19
SH	S11	195	12.97 ± 4.42	10.93 ± 1.31	0.96 ± 0.11
	S12	95	13.27 ± 3.61	11.06 ± 1.07	0.96 ± 0.11
	S8	184	14.01 ± 4.81	11.11 ± 1.37	0.98 ± 0.10
	S13	176	16.09 ± 6.61	11.72 ± 1.69	0.95 ± 0.11

674

675

676

Table 2. Mean weight, length, and condition factor of *Salvelinus fontinalis* at 18 months. Line: C = control, S = selected; Family performance L = low, H = high; Phenotype: S = slow, F = fast; Group designation: line, family performance, phenotype; n = number of individuals.

Line	Family performance	Phenotype	Group	n	Weight (g)	Length (cm)	Condition
Control	Low	Slow-growing	CLS	32	5.03 ± 1.39	8.18 ± 0.71	0.90 ± 0.05
		Fast-growing	CLF	32	12.91 ± 3.03	11.24 ± 0.82	0.90 ± 0.11
	High	Slow-growing	CHS	32	6.95 ± 1.04	9.11 ± 0.48	0.92 ± 0.11
		Fast-growing	CHF	32	19.06 ± 3.85	12.90 ± 0.78	0.88 ± 0.09
Selected	Low	Slow-growing	SLS	32	6.33 ± 0.95	8.59 ± 0.54	1.00 ± 0.09
		Fast-growing	SLF	32	18.57 ± 3.42	12.49 ± 0.87	0.95 ± 0.10
	High	Slow-growing	SHS	32	6.62 ± 1.43	8.88 ± 0.62	0.94 ± 0.12
		Fast-growing	SHF	32	25.59 ± 11.18	13.99 ± 1.27	0.90 ± 0.09

Table 3. Information on sequences and primers obtained for *Salvelinus fontinalis*. For each studied gene, we present the species of origin of the sequences used to amplify the gene in brook charr, the designed PCR primers, the amplicon size (number of base pairs [bp]), and the percentage of similarity obtained between the original sequence and the *S. fontinalis* amplified sequence. Abbreviations are as follows: *grl*: ghrelin; *srifr*: somatostatin receptor; *lepr*: leptin receptor; *ghr-1*: growth hormone receptor 1; *igf-1*: insulin-like growth factor 1; *mhc*: myosin heavy chain.

Gene	Sequence used for primer design (accession no.)	Designed primers (5'-3')	<i>S. fontinalis</i> PCR amplicon size (bp)	<i>S. fontinalis</i> sequence similarity
<i>grl</i>	<i>Salvelinus alpinus</i> (XM_023995867)	F – ACTGATGCTGTGTACTCTGGC R – CTCTCAATGTCTCGCCGACC	223	97%
<i>srifr</i>	<i>Oncorhynchus mykiss</i> (NM_001124534)	F – GGGAAAAGACACCGGTTGGA R – TGGTGTGCGCTGTTAGACCC	273	98%
<i>lepr</i>	<i>Salvelinus alpinus</i> (XM_024004689)	F – CAGTTAGCTACATGTCGGGGA R – GCCGATTTCCCAGTAGCTGA	209	97%
<i>ghr-1</i>	<i>Oncorhynchus mykiss</i> (AY861675)	F – TTGCTGATACGGGTCGAACAT R – GAGGGTCTGGTTCCACGATG	431	99%
<i>igf-1</i>	<i>Oncorhynchus mykiss</i> (M95183.1)	F – TCAAGAGTGCATGTGCTGT R – TTCGGTAGTTCCTTCCCCCT	301	100%
<i>mhc</i>	<i>Salvelinus alpinus</i> (XM_023984421)	F – GTTGAAGATCCGAGTGCAGGT R – CGGGAACAGCTCAGGGATAAC	506	99%

Table 4. qPCR IDT assays used for transcriptomics in *Salvelinus fontinalis* pituitary. Genes (*18s*: 18s ribosomal; *β -actine*: beta actin; *efl- α* : elongation factor 1 alpha; *gh*: growth hormone; *ghr-1*: *gh* receptor 1; *igf-1r*: *igf-1* receptor; *grl*: ghreline; *srifr*: somatostatin), primers (5'-3') (F [forward], R [reverse], P [probe]), bp (number of base pairs), and Tm (melting temperature; °C) are given.

Gene	Primer (5'-3')	bp	Tm
<i>18s</i>	F – CAAGACGAACGAAAGCGAAAG	21	62
	P – AACGAAAGTCGGAGGTTCTGAAGACG	25	68
	R – AGATACCGTCGTAGTTCCGA	20	62
<i>β-actine</i>	F – AGAGAGGTATCCTGACTCTGAAG	23	62
	P – CACCAACTGGGACGACATGGAGAA	24	68
	R – CATCACACCTTCCTACAACGAG	22	62
<i>efl-α</i>	F – ATCGGCGGTATTGGAACAG	19	62
	P – CCTGAAGGCCGGTATGATCGTCAC	24	68
	R – GTGAAGTCTGTGGAGATGCA	20	62
<i>gh</i>	F – GTCGCTAAGACAGGCTCTTG	20	62
	P – CGTCTACAGAGTGCAGTTGGCCTC	24	68
	R – AAGGTCGAGACCTACCTGAC	20	62
<i>ghr-1</i>	F – CCCACTGCCCCCTGTATCT	19	62
	P – CTCAGAAGGAGGCTGTTTTGC	22	71
	R – ACCATGGTGGAAAGGAG	16	50
<i>igf-1r</i>	F – CAGCCTCATCACTGTACTCTTC	22	61
	P – AAAGAGGAACAGTGACAGGCTGGG	24	68
	R – CTCAGGGTTGACAGAAGCATAG	22	61
<i>grl</i>	F – CCCAGAAACCACAGGGTAAA	20	61
	P – TTGGTCGGCGAGACATTGAAAGCT	24	68
	R – TTTGTCTTCCTGGTGAAGGG	20	61
<i>srifr</i>	F – CTTAGCTCACAGTAGGAGAAACC	23	62
	P – AATAGACAACATGGCCGCAATGG	24	67
	R – GACTAGCAACTACCCAGCATAAC	22	62

Table 5. qPCR TaqMan assays used for *Salvelinus fontinalis* transcriptomics in the brain, liver, and muscle. Genes (*18s*: 18s ribosomal; *β-actin*: beta actin; *efl-α*: elongation factor 1 alpha; *npy*: neuropeptide Y; *lepr*: leptin receptor; *igf-1*: insulin growth like factor 1; *ghr-1*: *gh* receptor 1; *igf-1r*: *igf-1* receptor; *mhc*: myosin heavy chain; *myo*: myogenin), primers (5'-3') (F [forward], R [reverse], P [probe]), bp (number of base pairs), and Tm (melting temperature; °C) are given.

Gene	Primer (5'-3')	bp	Tm
<i>18s</i>	F – GATCCATTGGAGGGCAAGTCT	21	59
	P – TGCCAGCAGCCGC	13	69
	R – GATACGCTATTGGAGCTGGAATTAC	25	58
<i>β-actin</i>	F – GGTCGTCCCAGGCATCAG	18	59
	P – ATGGTTGGGATGGGC	15	69
	R – CGTCTCCCACGTAGCTGTCTT	21	58
<i>eflα</i>	F – GCCCCTCCAGGATGTCTACA	20	59
	P – AATCGGCGGTATTGGA	16	69
	R – ACGGCCACGGGTACTG	17	59
<i>npy</i>	F – TGCTGAAGAGCTGGCCAAAT	20	60
	P – CTATACCCGCTCAGAC	17	70
	R – TCTGTCTCGTGATCAGATTGATGTAG	26	58
<i>lepr</i>	F – CAGCATTCTGACATTGCTTTAACA	24	58
	P – TATGGTCTACAACAGTAGCTT	21	68
	R – CACCAATTCAAGGGCGGATA	20	59
<i>igf-1</i>	F – CGGTCACATAACCGTGGTATTG	22	59
	P – CGAGTGCTGCTTCC	14	70
	R – GCCGCAGCTCGCAACT	16	59
<i>ghr-1</i>	F – CCCACTGCCCCCTGTATCT	19	62
	P – CTTCAGAAGGAGGCTGTTTTGC	22	71
	R – ACCATGGTGAAGGAG	16	50
<i>igf-1r</i>	F – TCCTCAGTGGGACCCTTCTG	20	59
	P – CCGCCGGACTATAG	14	69
	R – GGACCATGAAGCCCAGTAGGT	21	59
<i>mhc</i>	F – CAAACCACATTGAACACCATCAG	23	59
	P – CACCACACTAGAAGTGT	17	69
	R – GGGTTAAGCTTTATTGATACAGGAAGTG	28	60
<i>myog</i>	F – CCTTGGGCCTGCAAGCT	17	58
	P – TGCAAACGCAAGACT	15	69
	R – CGCTTTTCGTGGTCCAT	18	58

Table 6. Effect of body length, condition, and line (selected or control) on the relative expression of genes related to the growth regulation pathway in 1+ *Salvelinus fontinalis* in different sampled tissues (liver, brain, pituitary, and muscle). Only final linear models (LM) and linear mixed models (LMM) including at least one significant effect are presented in this table (final models containing no significant effects are presented as supplementary material). Total n = 96 for all tissues except pituitary (total n = 39). Family was included in all models except pituitary as a random effect. For pituitary, family was the statistical unit. Estimates in bold are significant. *ghr-1* = growth hormone receptor 1; *igf-1* = insulin-like growth factor 1; *lepr* = leptin receptor; *igf-1r* = insulin-like growth factor 1 receptor; *myog* = myogenin.

	Estimate	SE	Variance	P-value
Liver				
<i>ghr-1</i> ($R^2 = 0.136$)				
Condition	1.964	0.865		0.026
Length	0.064	0.037		0.09
Line (selected)	0.548	0.156		0.001
Family (random effect)			0.069	0.20
<i>igf-1</i> ($R^2 = 0.236$)**				
Condition	1.939	1.069		0.07
Length	0.148	0.044		0.001
Line (selected)	0.681	0.183		<0.001
Family (random effect)			0.075	0.35
Brain				
<i>lepr</i> (Marginal $R^2 = 0.000$; Conditional $R^2 = 0.223$)**				
Condition	0.149	1.140		0.92
Length	0.068	0.045		0.13
Line (selected)	0.219	0.297		0.43
Family (random effect)			0.219	0.019
Pituitary				
<i>igf-1r</i> ($R^2 = 0.141$) **				
Condition	4.062	3.922		0.31
Length	0.207	0.093		0.034
Line (selected)	0.095	0.372		0.80
Muscle				
<i>ghr-1</i> ($R^2 = 0.061$)**				
Condition	0.437	1.148		0.70
Length	0.117	0.048		0.016
Line (selected)	0.019	0.213		0.93
Family (random effect)			0.087	0.38
<i>igf-1r</i> ($R^2 = 0.049$)**				
Condition	0.352	1.211		0.77
Length	0.028	0.049		0.56
Line (selected)	0.442	0.202		0.031

Family (random effect)		0.001	1.00
<i>myog</i> (Marginal $R^2 = 0.000$, Conditional $R^2 = 0.160$)			
Condition	0.111	0.958	0.92
Length	0.040	0.039	0.31
Line (selected)	0.004	0.249	0.99
Family (random effect)		0.112	0.038

*A log transformation was applied to achieve normality

** A Box-Cox transformation was applied to achieve normality

Supplementary Table 1. Effect of body length, condition, and line (selected or control) on the relative expression of genes related to the growth regulation pathway in 1+ *Salvelinus fontinalis* in different sampled tissues (brain, pituitary, and muscle). Only linear models (LM) and linear mixed models (LMM) including no significant effects are presented in this table (final models containing significant effects are in Table 6). Total n = 96 for all tissues except pituitary (total n = 39). Family was included in all models as a random effect except for pituitary, for which family was the statistical unit. *npv* = neuropeptid Y; *srifr* = somatostatin receptor; *gh* = growth hormone; *ghr-1* = growth hormone receptor 1; *grl* = ghrelin; *mhc* = myosin heavy chain.

	Estimate	SE	Variance	P-value
Brain				
<i>npv</i> **				
Condition	0.595	1.171		0.61
Length	0.070	0.049		0.16
Line (selected)	0.145	0.217		0.51
Family (random effect)			0.112	0.24
Pituitary				
<i>srifr</i> *				
Condition	5.221	6.406		0.42
Length	0.112	0.151		0.46
Line (selected)	0.170	0.607		0.78
<i>gh</i>				
Condition	3.379	2.849		0.25
Length	0.068	0.068		0.33
Line (selected)	0.033	0.270		0.90
<i>ghr-1</i> **				
Condition	1.470	4.308		0.74
Length	0.095	0.105		0.37
Line (selected)	0.311	0.367		0.40
<i>grl</i>				
Condition	4.062	3.922		0.31
Length	0.207	0.093		0.034
Line (selected)	0.095	0.372		0.80
Family (random effect)			<0.001	1.00
Muscle				
<i>Mhc</i> **				
Condition	1.561	1.139		0.17
Length	0.035	0.050		0.49
Line (selected)	0.041	0.217		0.85
Family (random effect)			<0.001	1.00

*A log transformation was applied to achieve normality

** A Box-Cox transformation was applied to achieve normality