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

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

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3 Martínez-Silva Maria A.<sup>1</sup>, Dupont-Prinet Aurélie<sup>1</sup>, Houle Carolyne<sup>2</sup>, Vagner Marie<sup>3</sup>, Garant  
4 Dany<sup>2</sup>, Bernatchez Louis<sup>4</sup>, Audet Céline<sup>1</sup>

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

8 <sup>2</sup>Département de biologie, Université du Sherbrooke, Sherbrooke, QC, J1K 2R1, Canada

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

11 <sup>4</sup>Institut 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

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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

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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/m<sup>3</sup>), and then in 500 L tanks (200 per family; 1200 ind/tank,  
144 mean charge of 10.8 kg/m<sup>3</sup>).

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<sup>-3</sup>. 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 ( $\pm 0.1$  g) and  
163 measured ( $\pm 0.1$  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<sup>-1</sup>) 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  $\mu\text{L}^{-1}$ . 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  $\mu\text{L}^{-1}$ , separated into aliquots, and kept frozen at  $-20^{\circ}\text{C}$  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 µL reactions containing 12.5 µL of AmpliTaq Gold 360  
205 (Applied Biosystems), 0.5 µL of 360 GC enhancer (Applied Biosystems), 2.5 µL of cDNA, 1.25  
206 µL each of forward and reverse primer (20 mM), and 7 µL of nuclease-free H<sub>2</sub>O. 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 µL of

224 diluted cDNA, 5  $\mu$ L of TaqMan Fast Advanced Mix, 0.5  $\mu$ L of Custom TaqMan Gene Expression  
225 Assay, and 2.5  $\mu$ L of sterile water, for a total volume of 10  $\mu$ 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*,  *$\beta$ -actin*, and *efl $\alpha$* , 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 ( $0.93 \pm 0.14$  vs  $0.92 \pm 0.12$ ;  $F_{(1,4470)} =$   
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

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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

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**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 – TGGTGTTCCTGTTAGACCC	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;  *$\beta$ -actine*: beta actin; *efl- $\alpha$* : 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><math>\beta</math>-actine</i>	F – AGAGAGGTATCCTGACTCTGAAG	23	62
	P – CACCAACTGGGACGACATGGAGAA	24	68
	R – CATCACACCTTCCTACAACGAG	22	62
<i>efl-<math>\alpha</math></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 – ACCATGGTGGGAAGGAG	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 – GACTAGCAACTACCCAGCATAC	22	62

**Table 5.** qPCR TaqMan assays used for *Salvelinus fontinalis* transcriptomics in the brain, liver, and muscle. Genes (*18s*: 18s ribosomal;  *$\beta$ -actin*: beta actin; *efl- $\alpha$* : 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><math>\beta</math>-actin</i>	F – GGTCGTCCCAGGCATCAG	18	59
	P – ATGGTTGGGATGGGC	15	69
	R – CGTCTCCCACGTAGCTGTCTT	21	58
<i>efl<math>\alpha</math></i>	F – GCCCCTCCAGGATGTCTACA	20	59
	P – AATCGGCGGTATTGGA	16	69
	R – ACGGCCACGGGTACTG	17	59
<i>npy</i>	F – TGCTGAAGAGCTGGCCAAAT	20	60
	P – CTATACCGCCTCAGAC	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 – ACCATGGTGGGAAGGAG	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 – CGCTTTTCGTGCGTCCAT	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
<b>Liver</b>				
<i>ghr-1</i> ( $R^2 = 0.136$ )				
Condition	1.964	0.865		<b>0.026</b>
Length	0.064	0.037		0.09
Line (selected)	0.548	0.156		<b>0.001</b>
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		<b>0.001</b>
Line (selected)	0.681	0.183		<b>&lt;0.001</b>
Family (random effect)			0.075	0.35
<b>Brain</b>				
<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	<b>0.019</b>
<b>Pituitary</b>				
<i>igf-1r</i> ( $R^2 = 0.141$ ) **				
Condition	4.062	3.922		0.31
Length	0.207	0.093		<b>0.034</b>
Line (selected)	0.095	0.372		0.80
<b>Muscle</b>				
<i>ghr-1</i> ( $R^2 = 0.061$ )**				
Condition	0.437	1.148		0.70
Length	0.117	0.048		<b>0.016</b>
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		<b>0.031</b>

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	<b>0.038</b>

\*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
<b>Brain</b>				
<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
<b>Pituitary</b>				
<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		<b>0.034</b>
Line (selected)	0.095	0.372		0.80
Family (random effect)			<0.001	1.00
<b>Muscle</b>				
<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