Genetically based population divergence in overwintering energy mobilization in brook charr (*Salvelinus fontinalis*)

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Abstract

Investigating the nature of physiological traits potentially related to fitness is important towards a better understanding of how species and/or populations may respond to selective pressures imposed by contrasting environments. In northern species in particular, the ability to mobilize energy reserves to compensate for the low external energy intake during winter is crucial. However, the phenotypic and genetic bases of energy reserve accumulation and mobilization have rarely been investigated, especially pertaining to variation in strategy adopted by different populations. In the present study, we documented variation in several energy reserve variables and estimated their quantitative genetic basis to test the null hypothesis of no difference in variation at those traits among three strains of brook charr (*Salvelinus fontinalis*) and their reciprocal hybrids. Our results indicate that the strategy of winter energy preparation and mobilization was specific to each strain, whereby i) domestic fish accumulated a higher amount of energy reserves before winter and kept accumulating liver glycogen during winter despite lower feeding; ii) Laval fish used liver glycogen and lipids during winter and experienced a significant decrease in condition factor; iii) Rupert fish had relatively little energy reserves accumulated at the end of fall and preferentially mobilized visceral fat during winter. Significant heritability for traits related to the accumulation and use of energy reserves was found in the domestic and Laval but not in the Rupert strain. Genetic and phenotypic correlations also varied among strains, which suggested population-specific genetic architecture underlying the expression of these traits. Hybrids showed limited evidence of non-additive effects. Overall, this study provides the first evidence of a genetically based—and likely adaptive—population-specific strategy for energy mobilization related to overwinter survival.

Keywords

Heritability, non-additive effects, energy mobilization strategy, local adaptation, genetic and phenotypic correlation, fish physiology.

Introduction

Understanding the adaptive potential of populations is a central goal in evolutionary biology. While phenotypic plasticity may allow populations to adapt in the short-term, phenotypic evolution is necessary for the long-term persistence of populations (Gienapp et al. 2008; Visser 2008; Bjorklund et al. 2009; Hoffmann and Sgro 2011). This requires a sufficient genetic component of variance of fitness-related traits upon which selection may act (Falconer and Mackay 1996; Kellermann et al. 2006; Serbezov et al. 2010). While heritability of traits (h^2) is the most commonly used predictor of evolutionary potential (Falconer and Mackay 1996; Lynch and Walsh 1998), other parameters such as the coefficient of additive genetic variance of traits (CV_A) can provide further insight into the potential of organisms to respond to selection (Houle 1992; Hermida et al. 2002). Also, the amount of additive genetic variance and heritability of traits typically differs among populations (Visscher et al. 2008) and there is thus a growing interest in investigating intraspecific variation, specifically on important physiological traits, and its underlying genetic basis (Zamer et al. 1999; Nespolo et al. 2003; Ronning et al. 2007; Tieleman et al. 2009). Such investigations are essential to assess if an adaptive response could be expected and the degree to which such response might differ among populations of a same species.

Energy mobilization during the first winter of life is an important physiological trait in populations from temperate climates since it could crucially affect survival and thus population

73 dynamics and recruitment (Sogard 1997; Child and Laing 1998; Huss et al. 2008). Under cold
74 climatic conditions, the annual fluctuations in temperature and food productivity create cycles in
75 energy availability. These cycles induce periods of energy reserve accumulation and depletion in
76 many vertebrates (Xiang and Peichao 1990; Boutilier et al. 1997; Hutchings et al. 1999; Box et
77 al. 2010; Vollenweider et al. 2011). Because of low temperatures, short days, and limited food
78 access, winter is a critical period for survival, especially for juvenile life stages (Finstad et al.
79 2004; Altwegg et al. 2005; Pelletier et al. 2007; Robles et al. 2007). To compensate for the low
80 energy intake and to reduce mortality risk, organisms rely on their ability to mobilize endogenous
81 energy reserves (Boutilier et al. 1997; Schultz and Conover 1999; Pelletier et al. 2007; Heermann
82 et al. 2009). In many vertebrates, this ability to deal with winter constraints operate in a size-
83 dependent manner, with larger individuals being favoured due to the allometry of energy
84 metabolism (Suttie and Webster 1995; Cargnelli and Gross 1997; Schultz and Conover 1999;
85 Hodges et al. 2006; Heermann et al. 2009).
86
87 The general pattern of energy reserve mobilization in freshwater fish is characterized by the
88 depletion of glycogen (inducing a reduction of liver mass), followed by the use of perivisceral fat
89 and hepatic lipids, and finally by the depletion of tissue proteins (Collins and Anderson 1995;
90 Rios et al. 2006). A marked depletion of energy reserves can then have a critical impact on an
91 organism's health and survival probability (Eckmann 2004; Hodges et al. 2006; Huss et al. 2008;
92 Tattersall and Ultsch 2008). Yet, little is known about the genetic basis of such a strategy and
93 thus on the ability of populations to physiologically adapt to different selection pressures.
94
95 Intraspecific variations in behavioural or energetic strategies to cope with winter have been
96 documented in several species (Schultz and Conover 1997; Goto et al. 1999; Polo et al. 2007;

97 Tattersall and Ultsch 2008; Finstad et al. 2010). In these studies, the differences in behaviour or
98 energy storage and depletion were generally linked to local adaptations and followed latitudinal
99 clines, with northern populations being more tolerant to winter temperatures and more efficient
100 with regard to behaviour or energy processes (Schultz et al. 1998; Goto et al. 1999; Polo et al.
101 2007; Tattersall and Ultsch 2008; Finstad et al. 2010). Thus, strategies for building winter energy
102 reserves could also result from genetically based local adaptations (Schultz and Conover 1997;
103 Billerbeck et al. 2001; Polo et al. 2007; Finstad et al. 2010). However, since previous studies
104 have generally been conducted in the field, the distinction of genetic versus environmental or
105 plastic effects on these traits has remained difficult to disentangle (Hoffmann and Merilä 1999;
106 Stelkens et al. 2009). While the few studies conducted in a common environment suggested the
107 presence of a genetic basis for rates of energy accumulation (Schultz and Conover 1997), the
108 actual quantitative genetic basis of energy reserve accumulation and mobilization has rarely been
109 documented. Nevertheless, there is a growing interest in determining the heritability of body
110 composition traits in the context of selective breeding programs in fishes (Kause et al. 2002;
111 Neira et al. 2004; Tobin et al. 2006; Navarro et al. 2009; Saillant et al. 2009) and other
112 economically important vertebrates (Lo et al. 1992; Hickey et al. 2007).
113
114 Besides allowing an estimate of heritability, inter-strain hybridization can also provide
115 important information on the genetic basis of performance. When populations are genetically
116 closer and display significant heritabilities for traits of interest, hybrids can express additive
117 effects and show performance levels intermediate to those of parental lines. Conversely, when
118 populations are genetically divergent and adapted to their own environments, hybrids can express
119 non-additive effects due to complex genetic associations that can enhance (heterosis) or reduce
120 (outbreeding depression) performance (Falconer and Mackay 1996; Edmands 1999; Stelkens et

al. 2009). Non-additive effects have been observed for different physiological traits such as growth rate, survival, and other fitness-related traits, revealing evolutionary divergence among populations (Emlen 1991; Hotz et al. 1999; Rieseberg et al. 1999; Cooke et al. 2001; Tymchuk et al. 2007) including those of brook charr (Granier et al. 2011). However, the occurrence of non-additive effects underlying energy processes has never been investigated.

In this study, we investigate intraspecific strategies of energy mobilization in three strains of brook charr (*Salvelinus fontinalis*) and their reciprocal hybrids by documenting the phenotypic and genetic bases of traits related to the accumulation of energy reserves in fall and their use during the first winter of life. More specifically, the objectives were (i) to compare energy reserve accumulation and mobilization among strains during winter in order to determine how local adaptation might have shaped this trait, (ii) to estimate heritability and genetic correlations in traits related to energy reserves in a common environment, and (iii) to evaluate the importance of non-additive effects in the energy strategies.

Materials and methods

Details pertaining to strain origin, breeding design and family rearing are presented in details in Crespel et al. (2011). We thus only briefly summarize this information below.

Brook charr strains

Three genetically distinct strains of brook charr (Martin et al. 1997) were used as parental lines. The Laval strain originates from a wild population of anadromous brook charr from the Laval River (48°44'N; 69°05'W) on the north shore of the St. Lawrence estuary (QC, Canada). The fish

used were from third generation breeders produced in captivity at the Station aquicole of ISMER/UQAR (Rimouski, QC, Canada). The Rupert strain originates from a northern lacustrine freshwater resident wild population inhabiting the Rupert River system (51°05'N; 73°41'W) (QC, Canada). These third generation breeders were reared in captivity at the Laboratoire Régional en Sciences Aquatiques (LARSA, Université Laval, Québec, QC, Canada). The third group was a domestic freshwater strain that has been widely used by the Québec fish farming industry for more than a hundred years. It originates from two strains (Nashua and Baldwin), and breeders were obtained from the Pisciculture de la Jacques Cartier (Cap-Santé, QC, Canada).

Breeding design

Hybrid and purebred crosses were made from mid-November 2005 until the end of December 2005 at LARSA using eggs and milt obtained from the different fish rearing locations. Three purebred strains were produced: ♀ domestic × ♂ domestic ($D_{♀}D_{♂}$), ♀ Laval × ♂ Laval ($L_{♀}L_{♂}$), and ♀ Rupert × ♂ Rupert ($R_{♀}R_{♂}$). Five reciprocal hybrids were produced: $D_{♀}R_{♂}$, $D_{♀}L_{♂}$, $L_{♀}D_{♂}$, $L_{♀}R_{♂}$, and $R_{♀}L_{♂}$. It was not possible to obtain the $R_{♀}D_{♂}$ cross because of the temporal differences in sexual maturation between these two strains (October for domestic males and December for Rupert females). All breeders were used only once. For each cross, 10 full-sib families were obtained through single-pair mating.

Family rearing

During the first six months, i.e., from egg incubation (January) to exogenous feeding (June), families were kept separate in recirculating fresh water and reared in seven troughs, each of which was divided into twelve units at LARSA. Water temperature was maintained at 6°C during egg incubation and at 8°C after hatching. In June, families were transferred to nine 3 m^3 tanks,

169 with eight families pooled per tank. Prior to transfer, families from all cross types were randomly
170 assigned to the different pools, with cross type randomized in pools, and were marked to allow
171 for identification by different combinations of adipose and pelvic fin clippings. All families were
172 brought to the same fry stage by the end of the summer and maintained at 10°C in recirculating
173 fresh water. The photoperiod followed the natural seasonal cycle, and fish were fed according to
174 commercial charts. In September, fish were transferred to the Station aquicole ISMER/UQAR,
175 where they were reared in 10 0.5 m^3 indoors tanks, with six to eight families per tank depending
176 on the initial poolings made at LARSA, under natural temperature and photoperiod conditions in
177 running dechlorinated fresh water. Fish were fed daily (1% w/w ration) with commercial dry
178 pellets until water temperature decreased to 4°C (in January); fish were then fed twice a week.
179 Due to the limited number of fish in some families, the number of families used for this
180 experiment was 10 for L♀R♂, 9 for L♀D♂ and L♀L♂, 8 for D♀D♂, 7 for D♀L♂ and D♀R♂, 6 for
181 R♀L♂, and 5 for R♀R♂. A daily record of mortalities was made throughout the winter and the
182 relative mortality was determined for each family.
183
184 Sampling
185 Two samplings were performed during the first winter to evaluate energy mobilization among the
186 different crosses: one in December (water temperature at 7°C) and the second in March (water
187 temperature at 3°C) (Fig. 1). For each sampling, ten fish per family were sacrificed (total number
188 of fish sampled = 1220) by anaesthesis in MS 222 (0.16 g/L [3-aminobenzoic acid ethyl ester])
189 and their body mass (to the nearest 0.1 g) and fork length (0.1 cm) were measured. Condition
190 factor was estimated according to the equation (weight / $length^3$) × 100. The liver was excised,
191 weighed to determine the hepato-somatic index (HSI: liver weigh / body weight × 100), rapidly
192 frozen in liquid nitrogen, and stored at -80°C until further analysis. Visceral fat deposits were

193 collected, weighed, and expressed in percentage of body weight. One piece of epaxial dorsal
194 muscle was excised, weighed, and dried for 72 h at 70°C for the determination of water content.
195 Liver glycogen concentration was measured on fresh liver using the amyloglucosidase digestion
196 method (Carr and Neff 1984) followed by glucose concentration determination (QuantiChrom^{TM}
197 Glucose Assay kit, BioAssay Systems, USA); total liver lipid concentration was evaluated on
198 fresh liver using the phospho-vanillin method (Frings et al. 1972); and liver protein concentration
199 was determined on fresh liver using a protein dye binding method (Protein Assay kit, Biorad,
200 USA) according to Bradford (1976). Total liver energy content was estimated after conversion of
201 protein, total lipids, and glycogen concentrations to energy using conversion factors of 24 kJ/g,
202 38 kJ/g, and 17 kJ/g for proteins, lipids, and carbohydrates, respectively (Jobling 1993).
203
204 Statistical analyses
205 Data normality and homogeneity of variance were verified with Kolmogorov-Smirnov and
206 Brown-Forsythe tests, respectively. Muscle water content (rank), liver total energy content (log),
207 and survival index (arcsin) data had to be transformed to obtain normality. The different response
208 variables were analyzed using mixed models with cross-type, sampling time, and their interaction
209 fitted as fixed effects and full-sib families fitted as a random effect. The percentage of fish that
210 died during winter was analyzed using one-way ANOVA with cross-type as factor (n = 61
211 families). The presence of non-additive effects was determined by the presence of significant
212 differences between the mean trait values of each reciprocal hybrid compared to the mean traits
213 of both parental strains according to the model results (Bryden et al. 2004). The *a posteriori*
214 Tukey test was used for mean comparisons when possible or replaced by the Games and Howell
215 test when variances were not homogenous. Analyses were made using Statistica 7.0 version for
216 Windows (StatSoft, USA). A significance level of $\alpha = 0.05$ was used in all statistical tests.

217 Quantitative genetic analysis

218 Our breeding design was used to fit animal models (Lynch and Walsh 1998) based only on the
219 three pure strains (not the hybrid crosses) with the software ASReml (V 2.0; Gilmour et al. 2006).
220 Variance components for all traits in each purebred population were estimated by Restricted
221 Maximum Likelihood (REML) using the following model:

222 $$y = \mu + Months + G + e$$

223 where y is the phenotypic observation for each population in December and March, μ is the
224 overall mean, *Months* is the fixed effect of the sampling time, G is the random genetic effect
225 linked to the pedigree structure (full-sib families), and e is the random residual effect. The total
226 phenotypic variance (V_P) of each trait was decomposed into genetic variance (V_G) and residual
227 variance (V_R). The broad-sense heritability (H^2) for each trait was estimated as the ratio of the
228 estimated genetic variance to the total phenotypic variance ($H^2 = V_G/V_P$). A complementary
229 analysis using calculations of evolvability (Houle 1992) was also done. Overall, heritability and
230 evolvability estimates were comparable and positively correlated (r = 0.74; when assessed over
231 all traits and strains). Consequently, only the heritability results are presented and interpreted in
232 the manuscript.

233

234 Genetic and phenotypic correlations were also estimated in each purebred strain using bivariate
235 models between traits for which heritability was significant (10 bivariate models in the Domestic
236 strain, 10 bivariate models in the Laval strain, and one bivariate model in the Rupert strain) using
237 the relationship $r_G = COV_{Ai,j}/(V_{Ai}\ V_{Aj})^{½}$ and $r_P = COV_{Pi,j}/(V_{Pi}\ V_{Pj})^{½}$, respectively. Standard errors
238 for variance and covariance components as well as for heritabilities and genetic correlations were
239 also estimated by the bivariate models using the ASReml software. The statistical significance of
240 the estimated genetic variances and covariances in each population were tested by comparing the

full model with a constrained model in which the (co)variance was set to zero using a likelihood ratio test (against a chi-square distribution, where χ^2 = -2*difference in log likelihood). The statistical significance of estimate comparisons among populations were tested using a likelihood ratio test that compared a model including all three purebred strains, and where all variance components were estimated independently for each population, to a constrained model in which estimates were set to be equal among the three strains.

Results

Overwinter mortalities were low (mean of 2.4% with a range from 0.32% ± 0.12 in $D_{♀}L_{♂}$ to 5.60% ± 3.60 in $D_{♀}R_{♂}$) and similar among crosses ($F_{1,7}$ = 1.61, P > 0.05). However, the difference in the use of energy reserves during winter varied among cross-types (significant interactions between sampling time and cross-types for all variables measured). Tables 1 and 2 summarize the statistical results of all energy reserves. The percentage of total variance explained by the models varied from 5% (liver protein concentration) to 53% (total liver energy content).

Condition factor

In December, domestic fish had a significantly higher condition factor than those of the other two pure strains (Tables 1 and 3). Condition factors in hybrids were similar to parental lines for hybrids between the Laval and Rupert lines and to the leaner parental line in hybrids issued from the domestic line ($L_{♀}D_{♂}$, $D_{♀}L_{♂}$, and $D_{♀}R_{♂}$). The condition factor in March was significantly lower than in December in almost all cross-types. The only two exceptions were the $D_{♀}D_{♂}$, and $D_{♀}R_{♂}$ crosses, where condition factors in December and March were similar (Tables 1 and 3). The strongest decrease in condition was observed in the $L_{♀}L_{♂}$ cross-type (22% reduction in

March compared to December; Table 3), whereas the decreases observed in hybrids were intermediate compared to their parental lines (Table 3).

Body reserves

Domestic fish had more visceral fat in December than the Laval and Rupert fish (Tables 1 and 3). Hybrids generally accumulated amounts of visceral fat similar to the parental line that accumulated the higher amount of fat, suggesting a possible dominance of the "fatty" phenotype. The $R_{♀}L_{♂}$ hybrid accumulated more visceral fat than either parental line, suggesting that there were non-additive effects for this trait (Table 3). The $R_{♀}R_{♂}$, $R_{♀}L_{♂}$, and $L_{♀}R_{♂}$ crosses were the only ones to significantly deplete their visceral fat during winter (Tables 1 and 3).

In both December and March, domestic fish had the lowest muscle water content, Laval fish had the highest, and Rupert fish were intermediate (Tables 1 and 3). In hybrids, muscle water content was always similar to the parental line that showed the lower muscle water content, suggesting dominance of this phenotype, except for the $D_{♀}R_{♂}$ hybrid, which was intermediate (see Table 3). December and March results were similar except for the $L_{♀}R_{♂}$ hybrid, which had higher muscle water content in March (Table 3). This increase of muscle water content in hybrids, with no change in their parental lines, indicates the presence of non-additive effects (Table 3).

In December, domestic and Laval fish had significantly higher HSI than the Rupert individuals (Tables 1 and 3). HSI in hybrids was always intermediate to that of the parental lines, suggesting additive effects for this trait (Table 3). In March, the HSI was higher than in December in the domestic strain while the reverse trend was observed in the Laval strain (Table 3). No difference was observed among sampling periods in the Rupert strain (Table 3). For hybrids, the seasonal

289 variation was intermediate ($L_{♀}D_{♂}$, $D_{♀}L_{♂}$) or similar (hybrids issued from the Rupert line) to that
290 of the parental lines (Table 3).
291
292 Liver reserves
293 In December, domestic fish had significantly more glycogen per gram of liver than those from
294 the Laval strain with Rupert fish being intermediate (Tables 2 and 4). Hybrids had relative liver
295 glycogen concentrations similar to their maternal line (Table 4). Non-additive effects were
296 present in the $D_{♀}R_{♂}$ cross-type, with glycogen concentration being lower than in fish from either
297 parental line (Table 4). Relative liver glycogen increased during winter in fish from the domestic
298 strain while Laval fish used this energy reserve, as indicated by a significant decrease in March
299 (Table 4). No overall change was observed in Rupert fish (Table 4). Hybrids from the Laval and
300 the Rupert lines showed results similar to the Rupert strain, and the $D_{♀}L_{♂}$ hybrid had an
301 intermediate response compared to its parental lines, with no difference between relative
302 glycogen concentration in December and March. The $L_{♀}D_{♂}$ and $D_{♀}R_{♂}$ hybrids had overall winter
303 variations similar to the domestic strain, with an increase of liver glycogen (Table 4).
304
305 Relative liver protein concentrations (mg/g of liver) were similar among all cross-types in
306 December (Tables 2 and 4). No change in relative liver protein was observed over winter for any
307 of the purebred crosses (Table 4). However, the $D_{♀}R_{♂}$ and $L_{♀}D_{♂}$ hybrids showed a significantly
308 lower protein concentration in March than in December (Table 4), suggesting the presence of
309 non-additive effects. All others hybrids were similar to their parental lines (Table 4).
310
311 Domestic fish had significantly higher relative total liver lipid concentration (mg/g of liver) in
312 December than the Laval and Rupert fish (Tables 2 and 4). The relative liver lipid concentration

313 in hybrids was generally not significantly different from those of their parental lines. The only
314 exception was the D♀R♂ hybrids, which were closest to the paternal Rupert line (Table 4). At the
315 end of winter, only the L♀L♂ cross-type showed a significant decrease in relative liver total lipid
316 concentration while no change was observed in the two other purebred crosses (Table 4). The
317 L♀R♀ hybrids also expressed a decrease in relative liver lipid concentration (Table 4). No change
318 in liver lipid content was observed in the other hybrids.
319
320 The total liver energy content was significantly higher in December in domestic fish than in
321 Laval and Rupert strains (Table 4). Most of the hybrids had intermediate total liver energy
322 content compared to their parental lines, except for the R♀L♂ and L♀R♂ hybrids, which were both
323 similar to the R♀R♂ cross-type (Table 4). The pure Laval strain was the only strain for which the
324 liver energy content was lower in March than in December (Table 4). All other pure and hybrid
325 cross-types showed no change in their liver energy content, suggesting dominance of the "high
326 energy" phenotype in hybrids.
327
328 Genetic effects and heritability
329 Significant genetic variances were observed for all traits (Table 5) except for liver protein and
330 lipid concentrations. However, there were notable differences among strains. In the domestic
331 strain, genetic variances for visceral fat, muscle water content, HSI, and liver total energy content
332 were all significant and showed medium to high values of heritability (see Table 5). In the Laval
333 strain, there was a significant genetic variance with medium heritability for the condition factor
334 (H^2 = 0.32), visceral fat (H^2 = 0.31), HSI (H^2 = 0.26), and relative liver glycogen concentration
335 (H^2 = 0.42) (Table 5). In contrast, condition factor for the Rupert strain was the only trait for
336 which a significant genetic component of variance was found and for which heritability was high

(H^2 = 0.50) (Table 5). Finally, the parameters for visceral fat and liver total energy content were significantly different among strains (Table 5).

Genetic and phenotypic correlations

Significant genetic covariances and phenotypic correlations were present between fish body mass and energy reserves, but again these differed among strains (Table 6). In domestic fish, significant genetic covariances and strong correlations were obtained between body mass and two energy reserve indices—visceral fat (r_G = 0.75) and total liver energy content (r_G = 0.99) (Table 6). These two energy reserves were also highly correlated to each other (r_G = 0.69; Table 6). No significant genetic covariances were detected between body mass and any of the energy reserve traits in the Laval strain (Table 6). However, in the same strain, significant genetic covariances and high genetic correlations (r_G > 0.90) were observed between condition factor and two energy reserve indices, i.e., visceral fat and relative liver glycogen (Table 6). These two energy reserve indices were also highly correlated with each other and with HSI (r_G of 0.94, 0.88, and 0.83 for relative liver glycogen vs. visceral fat, visceral fat vs. HSI, and HSI vs. relative liver glycogen, respectively) (Table 6). In the Rupert strain, covariance between body mass and condition factor was not significant (COV_A = -0.01).

Discussion

The main objective of this study was to test for the existence of genetically based differences in energy accumulation and mobilization among strains of brook charr. Winter survival was comparable among strains, although each of them coped with winter conditions using different energy strategies (Fig. 2). A genetic basis was detected for traits related to body condition and

361 energy storage/use in the domestic and Laval strains but not in the Rupert. Genetic and
362 phenotypic correlations also varied among strains, which was therefore suggestive of population
363 specific genetic architecture underlying the expression of these traits. Hybrids showed limited
364 evidence of non-additive effects. Overall, this study provides strong evidence for a genetically
365 based - and likely adaptive - population-specific strategy for energy mobilization related to
366 overwinter survival.
367
368 Different strains exhibited different genetically based energy strategies to cope with
369 constraints imposed by low winter temperature (see also Crespel et al. in press). Domestic fish
370 accumulated high amount of energy reserves before winter and kept accumulating liver glycogen
371 during winter despite the lower amount of food being available compared to other seasons.
372 Hepatic glycogen reserves play an important role in fish metabolism, and glycogen is the first
373 form of energy that is accumulated after starvation (Rios et al. 2006; Heermann et al. 2009).
374 Therefore, the domestic strain seemed to be relatively unaffected by low winter temperature
375 conditions. Laval fish had low energy reserves at the onset of winter and seemed to have suffered
376 energy costs during the coldest months since their condition factor was the most reduced by
377 March. Anadromous Laval fish, like most anadromous salmonids, have a low condition factor
378 and are more streamlined than their freshwater counterparts (Morinville and Rasmussen 2008).
379 Therefore, the observed decrease in condition factor during winter may reflect the cost related to
380 limited energy storage prior to winter. Anadromous fish overwinter away from their main feeding
381 area and therefore may not be adapted to feed and accumulate energy during winter. Yet,
382 mortality was no greater in Laval fish than in the other cross-types surveyed. During winter, the
383 strategy of the Laval fish was thus to mobilize liver glycogen and lipids, which is similar to the
384 general pattern of energy mobilization during starvation or limited energy intake in other fishes

385 (golden perch, *Macquaria ambigua*, Collins and Anderson 1995; Traira, *Hoplias malabaricus*,
386 Rios et al. 2006). In contrast, the strategy of Rupert brook charr, which also had relatively low
387 energy reserves accumulated by the end of autumn, was to mobilize visceral fat during winter,
388 which is compatible with the fact that these non-migratory fish overwinter in their main feeding
389 area which could be used during the winter. A similar preferential use of visceral fat has been
390 seen in starved gilthead seabream (*Sparus auratus*; Ibarz et al. 2010). To our knowledge, such
391 difference in energy mobilization strategies among populations has never been documented
392 previously in fishes. For aquatic organisms, perhaps the most comparable study to ours was
393 performed in oysters, whereby it was observed that one species (*Ostrea edulis*) preferentially
394 used lipids while the other (*Crassostrea* gigas) used protein reserves at low winter temperatures
395 (Child and Laing 1998). However, the genetic basis of those differences was not documented.
396
397 Our results suggest that brook charr could potentially adapt their energy mobilization strategy
398 in the long-term by evolutionary adjustments. The significant correlation observed between
399 heritability and evolvability estimates strengthen our conclusions. Studies on other animal species
400 have also revealed the presence of a significant genetic basis underlying energy mobilization as
401 well as substantial levels of additive variance in energy traits (H^2, Jones et al. 1992; H^2, Ronning
402 et al. 2007; narrow-sense heritability (h^2), Tieleman et al. 2009; H^2, Jumbo-Lucioni et al. 2010).
403 In fishes in particular, our heritability values for condition factor, muscle water content, and
404 visceral fat were generally in the upper range of estimates documented in species (condition
405 factor: 0.10–0.40; muscle water content: 0.06–0.36; visceral fat: 0.18–0.68; h^2, Kause et al. 2002;
406 H^2, Neira et al. 2004; H^2, Tobin et al. 2006; h^2, Navarro et al. 2009; h^2, Saillant et al. 2009).
407 Admittedly, such high values could be partially due to our full-sib design, which can
408 overestimate the genetic variance by including other variance sources (common environment

409 and/or maternal variance, and some portion of dominance variance) in addition to the additive
410 variance (Falconer and Mackay 1996; Perry et al. 2004). However, it is noteworthy that most
411 previous studies mentioned above also used full-sib families to estimate heritabilities. Our study
412 is however the first to report heritability values for liver energy reserves which varied widely
413 among strains. In addition, genetic correlations for some of the traits measured here were
414 consistent with those previously observed for other species, such as the rainbow trout
415 (*Oncorhynchus mykiss*), gilthead seabream, and sea bass (*Dicentrarchus Labrax*), for which
416 estimates varied from low to highly significant depending on the traits (r_G between condition
417 factor and muscle water content: -0.27–0.51; r_G between condition factor and visceral fat: 0.19–
418 0.87; r_G between muscle water content and visceral fat: 0.08–0.41; Kause et al. 2002; Navarro et
419 al. 2009; Saillant et al. 2009). Such correlations reflect the potential for the partially dependent
420 evolution of these traits.
421
422 Traits related to energy reserves were generally heritable in brook charr, although each strain
423 seemed to have its own characteristics. In the domestic and Laval strain strategies, energy
424 reserves had heritable genetic bases while the strategy of the Rupert strain did not. Moreover, two
425 traits related to energy reserves (visceral fat and total liver energy) had significantly different
426 heritabilities among the strains, revealing a major divergence in the underlying genetic basis of
427 these traits. The three strains also showed distinct patterns of genetic correlations among the
428 heritable traits measured, which is suggestive of differences in their genetic architecture related to
429 energy mobilization (Jensen et al. 2003; Robinson et al. 2009). The genetic basis for energy
430 strategy thus seems to be population-specific and cannot be extrapolated to other populations.
431 Our results also indicate that the three strains have distinct potential of physiological, and
432 possibly adaptive response to selective pressures associated with overwintering in a cold

433 environment. This is in contrast with the other few studies that compared the genetic basis of
434 energy-related traits. For instance, no difference in heritability or genetic correlation for energy
435 related-traits was observed among populations of coho salmon, *O. kisutch,* produced for
436 aquaculture (H^2, Neira et al. 2004) or among wild populations of stonechats, *Saxicola torquata*,
437 (h^2, Tieleman et al. 2009).
438
439 We found no evidence for a genetic basis related to the use of energy reserves in the Rupert
440 strain, suggesting that environmental effects are mainly involved in the observed phenotypic
441 variation of this strain. Traits submitted to strong directional selection pressure over a long time
442 are predicted to show eroded genetic variance due to fixation of beneficial alleles (Uller et al.
443 2002; Teplitsky et al. 2009). In the Rupert strain, traits related to energy reserves may thus have
444 been shaped by strong local selection that drastically reduced heritability. Similar results of low
445 heritable variation have been observed for physiological traits related to energy in mice (Nespolo
446 et al. 2003) and birds (Polo et al. 2007).
447
448 Divergences observed here in genetic architecture and strategies in energy accumulation and
449 mobilization could hypothetically be attributed to the adaptive response to distinct natural
450 environments (Collins and Anderson 1995; Roff and Mousseau 1999; Charmantier et al. 2004;
451 Hurst 2007). Although this remains to be more rigorously investigated, some ecological
452 differences among the three strains are worth mentioning. Namely, the Laval strain is
453 anadromous and originates from a population that migrates from freshwater for reproduction and
454 overwintering to saltwater in summer for feeding. The Rupert strain originates from a northern
455 lacustrine population subjected to harsh and long winter conditions. In contrast, the domestic
456 strain has been reared under artificial environments for about 100 years. Differences in

457 fluctuations of food abundance and winter conditions in these environments could thus have
458 influenced the strategies of energy storage and metabolism among strains. Clearly, a possible
459 causal link between differences among populations with different ecological conditions and
460 genetically based strategies of energy mobilization deserves further investigation.
461
462 Overall, we observed a limited occurrence of non-additive effects in hybrids. Non-additive
463 effects previously reported in hybrids mostly occurred when parental lines were divergent and
464 adapted to their own environments (Edmands 1999; Stelkens et al. 2009). In the present study, we
465 used purebred strains that are genetically very distinct both from a neutral (Martin et al. 1997)
466 and a functional (Bougas et al. 2010) standpoint. Therefore, the occurrence of non-additive
467 effects was expected, especially negative ones that would translate into outbreeding depression
468 (Bieri and Kawecki 2003). Although mainly expected to occur in later hybrid generations,
469 outbreeding depression may occur in the first generation, when the genetic composition of
470 populations is sufficiently divergent (Edmands 1999; Cooke et al. 2001; Tymchuk et al. 2007).
471 Moreover, non-additive effects on growth and gene expression have been reported for these same
472 three brook charr strains (Bougas et al. 2010; Granier et al. 2011). Bieri and Kawecki (2003)
473 indicated that small non-additive effects among genetically divergent populations may suggest
474 the absence of divergent evolution in coadapted gene complexes. This, and other possible effects
475 related to genetic architecture (e.g., pleitropy or other genetic linkage) deserve further rigorous
476 investigation.
477
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479

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484

485 **References**

486

487 Altwegg R, Dummermuth S, Anholt BR, Flatt T (2005) Winter weather affects asp viper *Vipera*
488 *aspis* population dynamics through susceptible juveniles. Oikos 110: 55-66.
489 Bieri J, Kawecki TJ (2003) Genetic architecture of differences between populations of cowpea
490 weevil (*Callosobruchus maculatus*) evolved in the same environment. Evolution 57: 274-
491 287.
492 Billerbeck JM, Lankford TE, Conover DO (2001) Evolution of intrinsic growth and energy
493 acquisition rates. I. Trade-offs with swimming performance in *Menidia menidia*.
494 Evolution 55: 1863-1872.
495 Bjorklund M, Ranta E, Kaitala V, Bach LA, Lunderberg P *et al.* (2009) Quantitative trait
496 evolution and environmental change. PLoS ONE 4: e4521. doi: 10.1371
497 Bougas B, Granier S, Audet C, Bernatchez L (2010) The transcriptional landscape of cross-
498 specific hybrids and its possible link with growth in brook charr (*Salvelinus fontinalis*
499 Mitchill). Genetics 186: 97-107. doi: 10.1534/genetics.110.118158
500 Boutilier RG, Donohoe PH, Tattersall GJ, West TG (1997) Hypometabolic homeostasis in
501 overwintering aquatic amphibians. J. Exp. Biol. 200: 387-400.
502 Box J, Lill A, Baldwin J (2010) Is there seasonal variation in stored energy reserves and catabolic
503 enzyme activities in Superb fairy-wrens? Avian Biol. Res. 3: 107-114.

504 Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities
505 of protein utilizing the principle of protein dye binding. Anal. Biochem. 72: 248-254.
506 Bryden CA, Heath JW, Heath DD (2004) Performance and heterosis in farmed and wild Chinook
507 salmon (*Oncorhynchus tshawyacha*) hybrid and purebred crosses. Aquaculture 235: 249-
508 261. doi: 10.1016/j.aquaculture.2004.01.027
509 Cargnelli LM, Gross MR (1997) Fish energetics: Larger individuals emerge from winter in better
510 condition. Trans. Am. Fish. Soc. 126: 153-156.
511 Carr RS, Neff JM (1984) Quantitative semi-automated enzymatic assay for tissue glycogen.
512 Comp. Biochem. Physiol. 77B: 447-449.
513 Charmantier A, Kruuk LEB, Blondel J, Lambrechts MM (2004) Testing for microevolution in
514 body size in three blue tit populations. J. Evol. Biol. 17: 732-743. doi: 10.1111/j.1420-
515 9101.2004.00734.x
516 Child AR, Laing I (1998) Comparative low temperature tolerance of small juvenile European,
517 *Ostrea edulis* L., and Pacific oysters, *Crassostrea gigas* Thunberg. Aquacult. Res. 29:
518 103-113.
519 Collins AL, Anderson TA (1995) The regulation of endogeneous energy stores during starvation
520 and refeeding in the somatic tissues of the golden perch. J. Fish Biol. 47: 1004-1015.
521 Cooke SJ, Kassler TW, Phillipp DP (2001) Physiological performance of largemouth bass related
522 to local adaptation and interstock hybridization: implications for conservation and
523 management. J. Fish Biol. 59: 248-268. doi: 10.1111/j.1095-8649.2001.tb01389.x
524 Crespel A, Bernatchez L, Garant D, Audet C (2011) Quantitative genetic analysis of the
525 physiological stress response in three strains of brook charr *Salvelinus fontinalis* and their
526 hybrids. J. Fish Biol. 79: 2019-2033. doi:10.1111/j.1095-8649.2011.03149.x

527 Crespel A, Bernatchez L, Audet C, Garant D (In press) Population specific gene-environment
528 interactions and evolutionary potential for growth in brook charr (*Salvelinus fontinalis*).
529 G3 Genes Genomes Genetics.
530 Eckmann R (2004) Overwinter changes in mass and lipid content of *Perca fluviatilis* and
531 *Gymnocephalus cernuus*. J. Fish Biol. 65: 1498-1511. doi: 10.1111/j.1095-
532 8649.2004.00553.x
533 Edmands S (1999) Heterosis and outbreeding depression in interpopulation crosses spanning a
534 wide range of divergence. Evolution 53: 1757-1768.
535 Emlen JM (1991) Heterosis and outbreeding depression - a multilocus model and an application
536 to salmon production. Fish. Res. 12: 187-212.
537 Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics. Longman Group, Essex,
538 UK.
539 Finstad AG, Berg OK, Forseth T, Ugedal O, Naesje TF (2010) Adaptive winter survival
540 strategies: defended energy levels in juvenile Atlantic salmon along a latitudinal gradient.
541 Proc. R. Soc. B-Biol. Sci. 277: 1113-1120.
542 Finstad AG, Ugedal O, Forseth T, Naesje TF (2004) Energy-related juvenile winter mortality in a
543 northern population of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 61: 2358-
544 2368. doi 10.1139/F04-213
545 Frings CS, Fendley TW, Dunn RT, Queen CA (1972) Improved determination of total serum
546 lipids by the sulfo-phospho-vanillin reaction. Clin. Chem. 18: 673-674.
547 Gienapp P, Teplitsky C, Alho JS, Mills JA, Merila J (2008) Climate change and evolution:
548 disentangling environmental and genetic responses. Mol. Ecol. 17: 167-178. doi:
549 10.1111/j.1365-294X.2007.03413.x

550 Gilmour AR, Gogel BJ, Cullis BR, Thompson R (2006) ASReml user guide release 2.0. VSN
551 International Ltd, Hemel Hempstead, UK.
552 Goto SG, Yoshida T, Beppu K, Kimura MT (1999) Evolution of overwinter strategies in Eurasian
553 species of the *Drosophila obscura* species group. Biol. J. Linn. Soc. 68: 429-441.
554 Granier S, Audet C, Bernatchez L (2011) Evidence for both heterosis and outbreeding depression
555 in growth of young-of-the year brook charr (*Salvelinus fontinalis*). Can. J. Zool. 89: 190-
556 198.
557 Heermann L, Eriksson LO, Magnhagen C, Borcherding J (2009) Size-dependent energy storage
558 and winter mortality of perch. Ecol. Freshwat. Fish 18: 560-571. doi: 10.1111/j.1600-
559 0633.2009.00371.x
560 Hermida M, Fernandes C, Amaro R, San Miguel E (2002) Heritability and "evolvability" of
561 meristic characters in a natural population of *Gasterosteus aculeatus*. Can. J. Zool. 80:
562 532-541.
563 Hickey JM, Keane MG, Kenny DA, Cromie AR, Veerkamp RF (2007) Genetic parameters for
564 EUROP carcass traits within different groups of cattle in Ireland. J. Anim. Sci. 85: 314-
565 321. doi: 10.2527/jas.2006-263
566 Hodges KE, Boonstra R, Krebs CJ (2006) Overwinter mass loss of snowshoe hares in the Yukon:
567 stravation, stress, adaptation or artefact? J. Anim. Ecol. 75: 1-13. doi: 10.1111/j.1365-
568 2656.2005.01018.x
569 Hoffmann AA, Merilä J (1999) Heritable variation and evolution under favourable and
570 unfavourable conditions. Trends Ecol. Evol. 14: 96-101.
571 Hoffmann AA, Sgro CM (2011) Climate change and evolutionary adaptation. Nature 470: 479-
572 485.

573 Hotz H, Semlitsch RD, Gutmann E, Guex GD, Beerli P (1999) Spontaneous heterosis in larval
574 life-history traits of hemiclonal frog hybrids. Proc. Natl. Acad. Sci. USA 96: 2171-2176.
575 Houle D (1992) Comparing evolvability and variability of quantitative traits. Genetics 130: 195-
576 204.
577 Hurst TP (2007) Causes and consequences of winter mortality in fishes. J. Fish Biol. 71: 315-345.
578 doi: 10.1111/j.1095-8649.2007.01596.x
579 Huss M, Bystrom P, Strand A, Eriksson LO, Persson L (2008) Influence of growth history on the
580 accumulation of energy reserves and winter mortality in young fish. Can. J. Fish. Aquat.
581 Sci. 65: 2149-2156. doi 10.1139/F08-115
582 Hutchings JA, Pickle A, McGregor-Shaw CR, Poirier L (1999) Influence of sex, body size, and
583 reproduction on overwinter lipid depletion in brook trout. J. Fish Biol. 55: 1020-1028.
584 Ibarz A, Blasco J, Gallardo MA, Fernandez-Borras J (2010) Energy reserves and metabolic status
585 affect the acclimation of gilthead sea bream (*Sparus aurata*) to cold. Comp. Biochem.
586 Phys. A 155: 319-326. doi: 10.1016/j.cbpa.2009.11.012
587 Jensen H, Saether BE, Ringsby TH, Tufto J, Griffith SC *et al.* (2003) Sexual variation in
588 heritability and genetic correlations of morphological traits in house sparrow (*Passer*
589 *domesticus*). J. Evol. Biol. 16: 1296-1307. doi: 10.1046/j.1420-9101.2003.00614.x
590 Jobling M (1993) Bioenergetics: feed intake and energy partitioning. In: J. C. RANKING and F. B.
591 JENSEN (ed) Fish ecophysiology,First edition. Chapman & Hall, London, pp. 1-44
592 Jones LD, Nielsen MK, Britton RA (1992) Genetic variation in liver mass, body mass, and liver -
593 body mass in mice. J. Anim. Sci. 70: 2999-3006.
594 Jumbo-Lucioni P, Ayroles JF, Chambers MM, Jordan KW, Leips J *et al.* (2010) Systems genetics
595 analysis of body weight and energy metabolism traits in *Drosophila melanogaster*. BMC
596 Genomics 11: 297.

Kause A, Ritola O, Paananen T, Mantysaari EA, Eskelinen U (2002) Coupling body weight and its composition: a quantitative genetic analysis in rainbow trout. Aquaculture 211: 65-79.

Kellermann VM, van Heerwaarden B, Hoffmann AA, Sgro CM (2006) Very low additive genetic variance and evolutionary potential in multiple populations of two rainforest *Drosophila* species. Evolution 60: 1104-1108. doi: 10.1554/05-710.1

Lo LL, McLaren DG, McKeith FK, Fernando RL, Novakofski J (1992) Genetic analyses of growth, real-time ultrasound, carcass, and pork quality in Duroc and Landrace pigs: II heritabilities and correlations. J. Anim. Sci. 70: 2387-2396.

Lynch M, Walsh JB (1998) Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland, MA.

Martin S, Savaria J-Y, Audet C, Bernatchez L (1997) Microsatellites reveal no evidence for inbreeding effects but low inter-stock genetic diversity among brook charr stocks used for production in Quebec. Bull. Aquacult. Assoc. Can. 97: 21-23.

Morinville GR, Rasmussen JB (2008) Distinguishing between juvenile anadromous and resident brook trout (*Salvelinus fontinalis*) using morphology. Environ. Bio. Fishes 81: 171-184. doi: 10.1007/s10641-007-9186-9

Navarro A, Zamorano MJ, Hildebrandt S, Gines R, Aguilera C *et al.* (2009) Estimates of heritabilities and genetic correlation for body composition traits and GXE interactions, in gilthead seabream (*Sparus auratus* L.). Aquaculture 295: 183-187. doi: 10.1016/j.aquaculture.2009.07.012

Neira R, Lhorente JP, Araneda C, Diaz N, Bustos E *et al.* (2004) Studies on carcass quality traits in two populations of Coho salmon (*Oncorhynchus kisutch*): phenotypic and genetic parameters. Aquaculture 241: 117-131. doi: 10.1016/j.aquaculture.2004.08.009

Nespolo RF, Bacigalupe LD, Bozinovic F (2003) Heritability of energetics in a wild mammal, the leaf-eared mouse (*Phyllotis darwini*). Evolution 57: 1679-1688.

Pelletier F, Reale D, Garant D, Coltman DW, Festa-Bianchet M (2007) Selection on heritable seasonal phenotypic plasticity of body mass. Evolution 61: 1969-1979. doi: 10.1111/j.1558-5646.2007.00160.x

Perry GML, Audet C, Laplatte B, Bernatchez L (2004) Shifting patterns in genetic control at the embryo-alevin boundary in brook charr. Evolution 58: 2002-2012.

Polo V, Carrascal LM, Metcalfe NB (2007) The effects of latitude and day length on fattening strategies of wintering coal tits *Periparus ater* (L.): a field study and aviary experiment. J. Anim. Ecol. 76: 866-872. doi: 10.1111/j.1365-2656.2007.01270.x

Rieserberg LH, Archer MA, Wayne RK (1999) Transgressive segregation, adaptation and speciation. Heredity 83: 363-372.

Rios FS, Moraes G, Oba ET, Fernandes MN, Donatti L *et al.* (2006) Mobilization and recovery of energy stores in traira, *Hoplias malabaricus* Bloch (Teleostei, Erythrinidae) during long-term starvation and after re-feeding. J. Comp. Physiol., B 176: 721-728. doi: 10.1007/s00360-006-0098-3

Robinson MR, Wilson AJ, Pilkington JG, Clutton-Brock TH, Pemberton JM *et al.* (2009) The impact of environmental heterogeneity on genetic architecture in a wild population of Soay sheep. Genetics 181: 1639-1648. doi: 10.1534/genetics.108.086801

Robles H, Ciudad C, Vera R, Baglione V (2007) No effect of habitat fragmentation on post-fledging, first-year and adult survival in the middle spotted woodpecker. Ecography 30: 685-694. doi: 10.1111/j.2007.0906-7590.05179.x

642 Roff DA, Mousseau TA (1999) Does natural selection alter genetic architecture? An evaluation
643 of quantitative genetic variation among populations of *Allonemobius socius* and *A.*
644 *fasciatus*. J. Evol. Biol. 12: 361-369.
645 Ronning B, Jensen H, Moe B, Bech C (2007) Basal metabolic rate: heritability and genetic
646 correlations with morphological traits in the zebra finch. J. Evol. Biol. 20: 1815-1822.
647 Saillant E, Dupont-Nivet M, Sabourault M, Haffray P, Laureau S *et al.* (2009) Genetic variation
648 for carcass quality traits in cultured sea bass (*Dicentrarchus labrax*). Aquat. Living
649 Resour. 22: 105-112. doi: 10.1051/Alr/2009010
650 Schultz ET, Conover DO (1997) Latitudinal differences in somatic energy storage: adaptive
651 responses to seasonality in an estuarine fish (Atherinidae: *Menidia menidia*). Oecologia
652 109: 516-529.
653 Schultz ET, Conover DO (1999) The allometry of energy reserve depletion: test of a mechanism
654 for size-dependent winter mortality. Oecologia 119: 474-483.
655 Schultz ET, Conover DO, Ehtisham A (1998) The dead of winter: size dependent variation and
656 genetic differences in seasonal mortality among Atlantic silverside (Atherinidae: *Menidia*
657 *menidia*) from different latitudes. Can. J. Fish. Aquat. Sci. 55: 1149-1157.
658 Serbezov D, Bernatchez L, Olsen EM, Vollestad LA (2010) Quantitative genetic parameters for
659 wild stream-living brown trout: heritability and parental effects. J. Evol. Biol. 23: 1631-
660 1641.
661 Sogard SM (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. Bull.
662 Mar. Sci. 60: 1129-1157.
663 Stelkens RB, Schmid C, Selz O, Seehausen O (2009) Phenotypic novelty in experimental hybrids
664 is predicted by the genetic distance between species of cichlid fish. BMC Evol. Biol. 9:
665 283. doi: 10.1186/1471-2148-9-283

666 Suttie JM, Webster JR (1995) Extreme seasonal growth in artic deer: comparisons and control
667 mechanisms. Am. Zool. 35: 215-221.
668 Tattersall GJ, Ultsch GR (2008) Physiological ecology of aquatic overwintering in ranid frogs.
669 Biol. Rev. 83: 119-140. doi: 10.1111/j.1469-185X.2008.00035.x
670 Teplitsky C, Mills JA, Yarrall JW, Merila J (2009) Heritability of fitness components in a wild
671 bird population. Evolution 63: 716-726. doi: 10.1111/j.1558-5646.2008.00581.x
672 Tieleman BI, Versteegh MA, Helm B, Dingemanse NJ (2009) Quantitative genetics parameters
673 show partial independent evolutionary potential for body mass and metabolism in
674 stonechats from different populations. J. Zool. 279: 129-136. doi: 10.1111/j.1469-
675 7998.2009.00597.x
676 Tobin D, Kause A, Mantysaari EA, Martin SAM, Houlihan DF *et al.* (2006) Fat or lean? The
677 quantitative genetic basis for selection strategies of muscle and body composition traits in
678 breeding schemes of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 261: 510-521.
679 doi: 10.1016/j.aquaculture.2006.07.023
680 Tymchuk WE, Sundstrom LF, Devlin RH (2007) Growth and survival trade-offs and outbreeding
681 depression in rainbow trout (*Oncorhynchus mykiss*). Evolution 61: 1225-1237. doi:
682 10.1111/j.1558-5646.2007.00102.x
683 Uller T, Olsson M, Stahlberg F (2002) Variation in heritability of tadpole growth: an
684 experimental analysis. Heredity 88: 480-484. doi: 10.1038/sj/hdy/6800088
685 Visscher PM, Hill WG, Wray NR (2008) Heritability in the genomics era - concepts and
686 misconceptions. Nat. Genet. 9: 255-266. doi: 10.1038/Nrg2322
687 Visser ME (2008) Keeping up with a warming world; assessing the rate of adaptation to climate
688 change. Proc. R. Soc. B Biol. Sci. 275: 649-659. doi: 10.1098/rspb.2007.0997

689 Vollenweider JJ, Heintz RA, Schaufler L, Bradshaw R (2011) Seasonal cycles in whole-body
690 proximate composition and energy content of forage fish vary with water depth. Mar.
691 Biol. 158: 413-427.
692 Xiang J, Peichao W (1990) Annual cycles of lipid contents and caloric values of carcass and
693 some organs of the gecko, *Gekko japonicus*. Comp. Biochem. Physiol. 96A: 267-271.
694 Zamer WE, McManus MG, Rowell CB (1999) Physiological variation in clonal anemones:
695 energy balance and quantitative genetics. Am. Zool. 39: 412-421.
696

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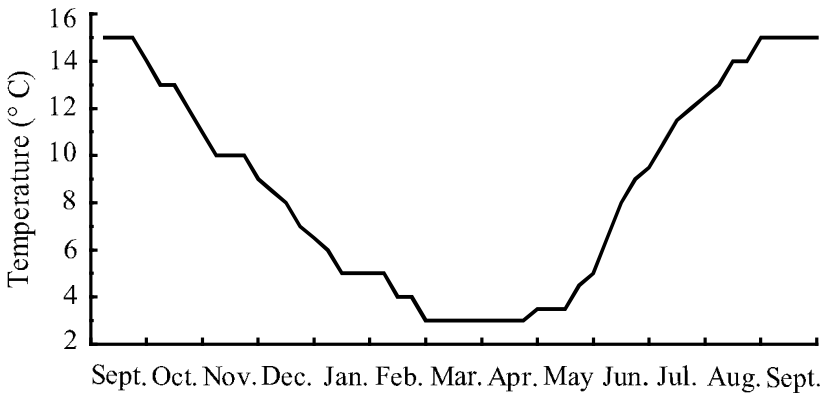

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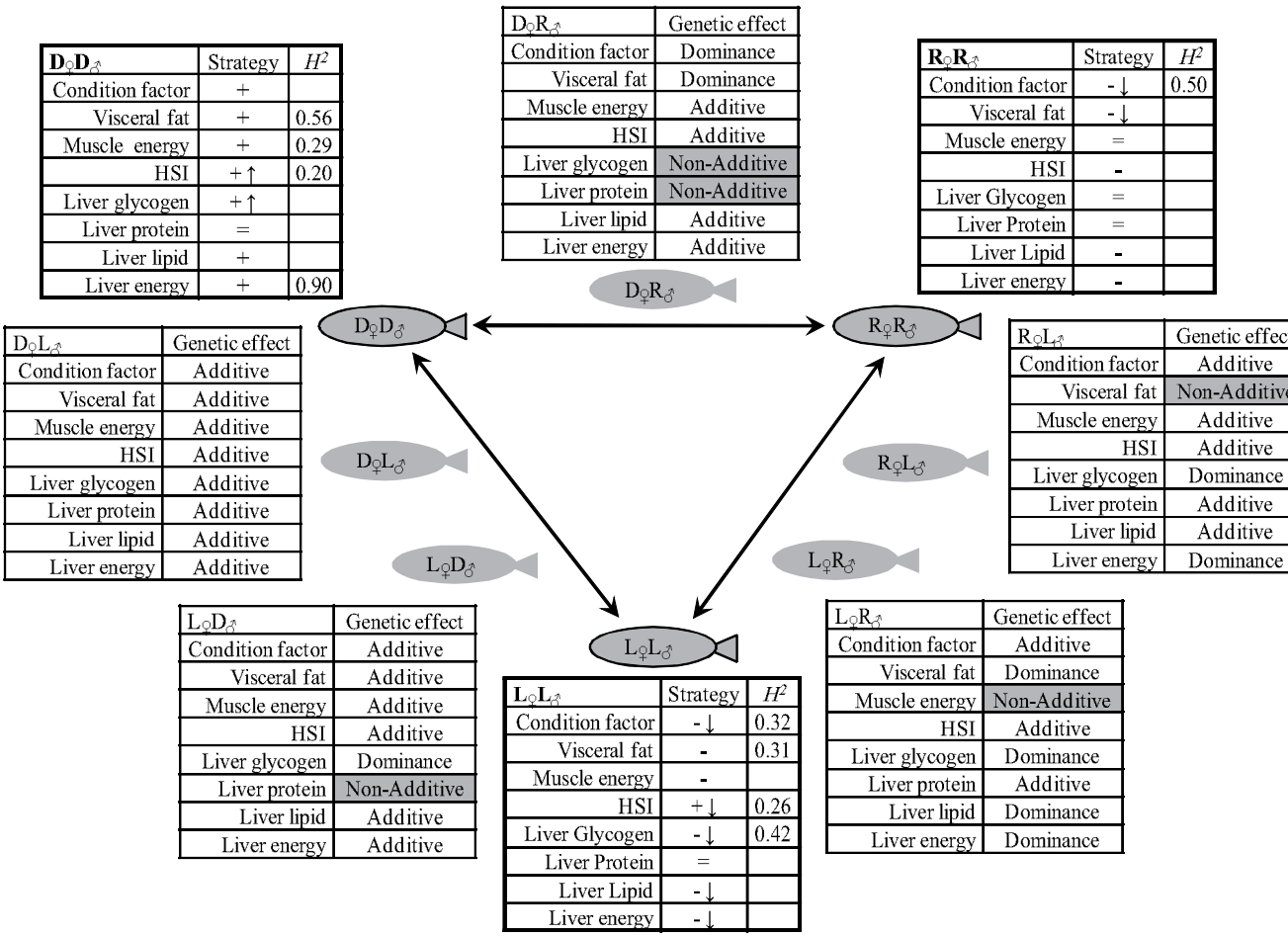

Tables
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Table 1: Summary of the mixed model statistics on sampling time × cross-type interactions for condition factor and traits related to body energy reserves (visceral fat, muscle water content, and HSI [hepato-somatic index]).

	Condition factor			Visceral fat (%)			Muscle water content (%)			HSI (%)		
	df	F	*P*-value	df	F	*P*-value	df	F	*P*-value	df	F	*P*-value
Sampling time	1	359.7	< 0.001	1	39.6	< 0.001	1	0.5	0.49	1	0.1	0.92
Cross-type	7	20.6	< 0.001	7	5.9	< 0.001	7	16.3	< 0.001	7	17.2	< 0.001
Sampling time × Cross-type	7	15.0	< 0.001	7	6.6	< 0.001	7	8.4	< 0.001	7	10.4	< 0.001
Family (nested in Cross-type), random	53	3.4	< 0.001	53	6.1	< 0.001	53	3.7	< 0.001	53	3.7	< 0.001
Residual error	1147			1147			1147			1147		
Model R^2	0.51			0.36			0.37			0.39		
Adjusted R^2	0.48			0.33			0.34			0.35		

Table 2: Summary of the mixed model statistics on sampling time × cross-type interactions for liver energy reserve traits (glycogen, protein, lipid, and total energy).

	Liver glycogen (mg/g)			Liver protein (mg/g)			Liver lipid (mg/g)			Total energy (kJ)		
	df	F	*P*-value	df	F	*P*-value	df	F	*P*-value	df	F	*P*-value
Sampling time	1	2.8	0.1	1	39.7	< 0.001	1	25.2	< 0.001	1	0.3	0.61
Cross-type	7	13.8	< 0.001	7	3.1	0.008	7	12.0	< 0.001	7	25.3	< 0.001
Sampling time × Cross-type	7	28.3	< 0.001	7	2.5	0.02	7	3.0	0.004	7	9.9	< 0.001
Family (nested in Cross-type), random	53	3.4	< 0.001	53	0.9	0.69	53	1.5	0.02	53	5.9	< 0.001
Residual error	1122			1124			1124			1121		
Model R^2	0.38			0.10			0.18			0.56		
Adjusted R^2	0.35			0.05			0.13			0.53		

Table 3: Body condition and energy reserves measured as condition factor, visceral fat (%), muscle water content (%), and HSI (hepato-somatic index; %) in the three purebred strains (bold) and their hybrids in December and March. Means ± SEM; n is the number of individuals. The different letters indicate significant differences among cross-types in December and March ($\alpha = 0.05$). In December, grey highlight indicates hybrids that had significantly more or less energy reserves than both parental lines (non-additive effects). In March, grey highlight indicates hybrids that used significantly more or less energy reserves than both parental lines during winter (non-additive effects).

	December					March				
Cross	n	Condition Factor	Visceral fat (%)	Muscle water (%)	HSI (%)	n	Condition Factor	Visceral fat (%)	Muscle water (%)	HSI (%)
D♀R♂	70	1.02 ± 0.01^{fg}	2.50 ± 0.12^{cde}	78.5 ± 0.14^{bcd}	1.35 ± 0.03^{c}	68	0.98 ± 0.02^{cdef}	2.41 ± 0.14^{cde}	78.0 ± 0.12^{ab}	1.47 ± 0.05^{c}
D♀D♂	**80**	**1.11 ± 0.01^{h}**	**2.84 ± 0.12^{de}**	**78.2 ± 0.11^{abc}**	**1.64 ± 0.03^{d}**	**80**	**1.07 ± 0.01^{gh}**	**2.32 ± 0.11^{cd}**	**77.8 ± 0.10^{a}**	**1.84 ± 0.05^{e}**
D♀L♂	70	1.05 ± 0.01^{fg}	2.90 ± 0.12^{e}	78.1 ± 0.07^{ab}	1.57 ± 0.03^{d}	70	0.94 ± 0.01^{cd}	2.39 ± 0.15^{cde}	78.3 ± 0.10^{abcd}	1.61 ± 0.04^{d}
L♀D♂	90	1.04 ± 0.01^{fg}	3.01 ± 0.31^{cde}	78.6 ± 0.12^{cde}	1.66 ± 0.04^{de}	90	0.93 ± 0.01^{c}	2.92 ± 0.15^{de}	78.3 ± 0.11^{abcd}	1.66 ± 0.04^{de}
L♀L♂	**90**	**0.99 ± 0.02^{def}**	**1.71 ± 0.11^{ab}**	**80.0 ± 0.31^{g}**	**1.63 ± 0.03^{d}**	**89**	**0.77 ± 0.01^{a}**	**1.62 ± 0.11^{ab}**	**79.9 ± 0.27^{g}**	**1.37 ± 0.02^{c}**
L♀R♂	100	0.98 ± 0.01^{de}	2.25 ± 0.09^{c}	78.7 ± 0.16^{cde}	1.35 ± 0.02^{c}	99	0.85 ± 0.01^{b}	1.40 ± 0.09^{a}	79.2 ± 0.10^{fg}	1.29 ± 0.02^{bc}
R♀L♂	60	1.00 ± 0.02^{def}	2.71 ± 0.13^{de}	78.6 ± 0.06^{ef}	1.33 ± 0.03^{bc}	60	0.87 ± 0.01^{b}	2.10 ± 0.15^{bc}	78.9 ± 0.14^{ef}	1.29 ± 0.04^{abc}
R♀R♂	**50**	**1.02 ± 0.01^{ef}**	**2.02 ± 0.14^{bc}**	**78.7 ± 0.27^{def}**	**1.18 ± 0.04^{a}**	**50**	**0.94 ± 0.01^{cd}**	**1.33 ± 0.10^{a}**	**78.7 ± 0.19^{cdef}**	**1.16 ± 0.03^{a}**

Table 4: Liver energy reserves measured as glycogen (mg/g), protein (mg/g), lipid (mg/g), and liver total energy (kJ) in the three purebred strains (bold) and their hybrids in December and March. Means ± SEM; n is the number of individuals. The different letters indicate significant differences among cross-types in December and March ($\alpha = 0.05$). In December, grey highlight indicates hybrids that had significantly more or less energy reserves than both parental lines. In March, grey highlight indicates hybrids that used significantly more or less energy reserves than both parental lines during winter (non-additive effects).

	December					March				
Cross	n	Glycogen (mg/g)	Protein (mg/g)	Lipid (mg/g)	Total energy (kJ)	n	Glycogen (mg/g)	Protein (mg/g)	Lipid (mg/g)	Total energy (kJ)
D♀R♂	67	45.5 ± 2.1^{bc}	58.7 ± 2.1^{c}	25.7 ± 1.2^{cde}	0.92 ± 0.06^{de}	66	71.2 ± 4.3^{fg}	48.5 ± 1.7^{ab}	26.1 ± 1.3^{cdef}	1.49 ± 0.14^{ef}
D♀D♂	**80**	**61.4 ± 2.1^{ef}**	**54.9 ± 1.6^{abc}**	**31.9 ± 1.1^{f}**	**1.72 ± 0.13^{fg}**	**79**	**91.2 ± 4.3^{g}**	**48.6 ± 1.6^{ab}**	**31.4 ± 1.5^{ef}**	**2.53 ± 0.19^{g}**
D♀L♂	67	56.8 ± 2.3^{def}	60.5 ± 1.9^{c}	27.2 ± 1.3^{cdef}	1.13 ± 0.08^{e}	69	65.3 ± 4.4^{def}	53.5 ± 1.6^{abc}	25.6 ± 1.2^{cde}	1.63 ± 0.18^{ef}
L♀D♂	88	50.1 ± 1.7^{cd}	57.5 ± 1.4^{c}	30.4 ± 1.3^{def}	1.21 ± 0.08^{e}	89	67.4 ± 3.5^{f}	47.7 ± 1.4^{a}	25.8 ± 1.1^{cd}	1.47 ± 0.12^{ef}
L♀L♂	**87**	**48.8 ± 2.2^{cd}**	**54.8 ± 1.4^{abc}**	**25.6 ± 1.1^{cde}**	**0.44 ± 0.02^{b}**	**87**	**19.8 ± 2.1^{a}**	**55.5 ± 1.6^{bc}**	**18.6 ± 0.8^{a}**	**0.26 ± 0.02^{a}**
L♀R♂	99	44.4 ± 1.6^{bc}	58.3 ± 1.5^{c}	24.9 ± 0.8^{c}	0.64 ± 0.03^{c}	97	35.0 ± 2.6^{b}	53.8 ± 1.7^{abc}	19.0 ± 0.8^{ab}	0.56 ± 0.05^{bc}
R♀L♂	59	61.1 ± 2.8^{def}	57.6 ± 1.7^{bc}	26.9 ± 1.4^{cdef}	0.72 ± 0.06^{cd}	59	48.8 ± 4.7^{bcde}	54.5 ± 1.9^{abc}	23.9 ± 1.3^{bc}	0.69 ± 0.07^{bcd}
R♀R♂	**49**	**57.4 ± 3.3^{def}**	**59.1 ± 2.4^{c}**	**24.2 ± 1.4^{abcd}**	**0.48 ± 0.04^{bc}**	**49**	**46.6 ± 4.0^{bcde}**	**55.2 ± 2.2^{abc}**	**22.7 ± 1.2^{abc}**	**0.48 ± 0.04^{bc}**

Table 5: Estimates of the broad-sense heritability (H^2 ± SE) and genetic variance (V_G ± SE) for condition factor and the different traits related to energy reserves in the three purebred populations. Significant values are in bold. Null estimates represent parameters being constrained. Significance of differences (Difference) in genetic variance among strains was obtained from a likelihood ratio test (see text for details).

	Domestic		Laval		Rupert		Difference
	H^2	V_G	H^2	V_G	H^2	V_G	*P*-value
Condition factor	0.09 ± 0.10	0.001 ± 0.001	**0.32 ± 0.17**	**0.005 ± 0.003**	**0.50 ± 0.31**	**0.004 ± 0.004**	0.065
Visceral fat	**0.56 ± 0.25**	**0.481 ± 0.290**	**0.31 ± 0.17**	**0.340 ± 0.217**	0	0	0.033
Muscle water	**0.29 ± 0.18**	**0.256 ± 0.178**	0.03 ± 0.06	0.188 ± 0.472	0	0	0.28
HSI	**0.20 ± 0.14**	**0.024 ± 0.019**	**0.26 ± 0.15**	**0.021 ± 0.014**	0.05 ± 0.11	0.003 ± 0.006	0.21
Liver glycogen	0.08 ± 0.09	74.83 ± 88.07	**0.42 ± 0.20**	**164.90 ± 98.71**	0.11 ± 0.14	71.48 ± 96.78	0.42
Liver protein	0	0	0.01 ± 0.06	2.50 ± 11.63	0	0	0.81
Liver lipid	0.06 ± 0.08	8.46 ± 11.98	0.13 ± 0.11	10.18 ± 9.01	0	0	0.18
Total energy	**0.90 ± 0.29**	**1.97 ± 1.12**	0.04 ± 0.07	0.001 ± 0.002	0.06 ± 0.11	0.004 ± 0.008	< 0.001

Table 6: Estimates of genetic (below diagonal) and phenotypic (above diagonal) correlations with standard error (± SE) and genetic covariance (in italic below diagonal and genetic correlations) for body mass, condition factor, and the different energy reserves for which heritabilities were significant in all the purebred strains using bivariate analyses. Significant genetic covariances (from the likelihood ratio test) are in bold and asterisks indicate marginally non-significant covariances.

	Mass	Condition factor	Visceral fat	Muscle water	HSI	Liver glycogen	Total energy
Domestic:							
Mass	—		**0.55 ± 0.11**	-0.42 ± 0.10*	0.20 ± 0.11		**0.90 ± 0.03**
Visceral fat	**0.75 ± 0.19**		—	-0.52 ± 0.08*	0.29 ± 0.10		**0.52 ± 0.10**
	(4.56 ± 3.09)						
Muscle water	-0.65 ± 0.27*		-0.62 ± 0.29*	—	-0.17 ± 0.09		-0.42 ± 0.09*
	(-2.91 ± 2.29)		*(-0.11 ± 0.09)*				
HSI	0.53 ± 0.36		0.15 ± 0.48	-0.46 ± 0.45	—		0.54 ± 0.08
	(0.73 ± 0.70)		*(0.01 ± 0.02)*	*(-0.02 ± 0.02)*			
Total energy	**0.99 ± 0.01**		**0.69 ± 0.22**	-0.66 ± 0.27*	0.67 ± 0.28		—
	(13.11 ± 7.35)		***(0.38 ± 0.24)***	*(-0.23 ± 0.18)*	*(0.07 ±0.06)*		

Laval:					
Mass	—	0.24 ± 0.08	0.58 ± 0.07	0.04 ± 0.08	-0.04 ± 0.09
Condition factor	0.06 ± 0.65	—	**0.31 ± 0.09**	0.19 ± 0.09	**0.38 ± 0.08**
	(0.01 ± 0.02)				
Visceral fat	-0.52 ± 0.76	**0.90 ± 0.16**	—	**0.43 ± 0.07**	**0.27 ± 0.09**
	(-0.12 ± 0.15)	***(0.02 ± 0.01)***			
HSI	-0.91 ± 0.56	0.52 ± 0.36	**0.88 ± 0.16**	—	**0.39 ± 0.08**
	(-0.05 ± 0.04)	*(0.01 ± 0.01)*	***(0.04 ± 0.02)***		
Liver glycogen	-0.78 ± 0.58	**0.96 ± 0.15**	**0.94 ± 0.14**	**0.83 ± 0.23**	—
	(-3.41 ± 3.28)	***(0.37 ± 0.24)***	***(3.44 ± 2.04)***	***(0.66 ± 0.48)***	
Rupert:					
Mass	—	-0.04 ± 0.14			
Condition factor	-0.10 ± 0.62	—			
	(-0.01 ± 0.06)				

Figures legends

Fig. 1 Annual temperature profile of fresh water at the Station aquicole of ISMER/UQAR in Rimouski (QC, Canada)

Fig. 2 Schematic summary of energy reserve mobilization strategies, broad-sense heritabilities (H^2) in the three purebred strains (bold), and the genetic effects observed in their hybrids. In purebred strains, "+" indicates significantly more energy than in the other strains in December, "-" indicates significantly less, "=" indicates similar or intermediate energy compared to the other strains in December, and arrows indicate the energy reserve that significantly changed during winter (the strain with "↑" indicates significantly more energy in March than in December and "↓" significantly less). For heritability (H^2), only significant heritabilities are mentioned. In hybrids, "Additive" indicates intermediate energy compared to parental lines (i.e., additive genetic effects), "Dominance" indicates energy similar of one of the parental lines, and grey highlight indicates non-additive genetic effects when hybrids had or used significantly more or less energy reserves than both parental lines.