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Corresponding Author: Céline Audet, PhD

Corresponding Author's Institution: Université du Québec à Rimouski

First Author: Amélie Crespel, Ph.D. Student

Order of Authors: Amélie Crespel, Ph.D. Student; Louis Bernatchez, Ph.D.; Dany Garant, Ph.D.; Céline Audet, PhD

Abstract: Selection for stress resistance to transport has been identified as a target for genetic improvement in fish production. However, few studies have investigated the potential use of heterosis (i.e., hybrid vigour) to improve this trait, as well as specifically testing if hybrids are less sensitive to stress exposure than their parental lines. Three strains (domestic [D], Laval [L], and Rupert [R]) of brook charr (*Salvelinus fontinalis*) and their reciprocal hybrids were submitted to transport stress to measure stress resistance. Primary (cortisol) and secondary (glucose, osmolality, and haematocrit) stress responses were measured for each cross. Significant heritabilities were observed for both levels of stress response, with $h^2 = 0.60 (\pm 0.20)$ for plasma cortisol and $0.61 (\pm 0.20)$ for plasma glucose. We observed strain differences whereby the Rupert strain was the least sensitive to stress at the primary and secondary levels. No heterosis was detected, and only one case of outbreeding depression was present. The outbreeding depression was observed in the $D_{\text{♀}}R_{\text{♂}}$ hybrid, which had a 27% increase of plasma glucose compared to parental strains. The $D_{\text{♀}}R_{\text{♂}}$ and $R_{\text{♀}}L_{\text{♂}}$ hybrids had more pronounced variations (increase or decrease) in plasma osmolality than their respective parental strains, but these variations were difficult to relate definitively with the potential secondary stress response. These results indicate a strong potential for genetic improvement in the stress response to transport with the use of purebred crosses while hybridization has little value in this regard.

1 **Quantitative genetic analysis of the physiological stress response in three strains of brook charr,**
2 ***Salvelinus fontinalis* (Mitchill), and their hybrids**

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5 A. Crespel*, L. Bernatchez†, D. Garant‡, C. Audet*¹

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8 * Institut des sciences de la mer de Rimouski (ISMER), Université du Québec à Rimouski (UQAR),
9 310 des Ursulines, Rimouski, QC, G5L 3A1, Canada; † Institut de Biologie Intégrative et des Systèmes
10 (IBIS), Pavillon Charles-Eugène-Marchand, 1030, Avenue de la Médecine, Local 1145, Université
11 Laval, Québec, QC, G1V 0A6, Canada; and ‡ Département de biologie, Faculté des Sciences,
12 Université de Sherbrooke, Sherbrooke, QC, J1K 2R1, Canada

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17 Running headline: Stress response in brook charr

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¹ Author to whom correspondence should be addressed. Tel.: +1 418 723 1986 ext. 1744; fax: +1 418 724 1842; email: celine_audet@uqar.qc.ca

ABSTRACT

Selection for stress resistance to transport has been identified as a target for genetic improvement in fish production. However, few studies have investigated the potential use of heterosis (i.e., hybrid vigour) to improve this trait, as well as specifically testing if hybrids are less sensitive to stress exposure than their parental lines. Three strains (domestic [D], Laval [L], and Rupert [R]) of brook charr (*Salvelinus fontinalis*) and their reciprocal hybrids were submitted to transport stress to measure stress resistance. Primary (cortisol) and secondary (glucose, osmolality, and haematocrit) stress responses were measured for each cross. Significant heritabilities were observed for both levels of stress response, with $h^2 = 0.60 (\pm 0.20)$ for plasma cortisol and $0.61 (\pm 0.20)$ for plasma glucose. We observed strain differences whereby the Rupert strain was the least sensitive to stress at the primary and secondary levels. No heterosis was detected, and only one case of outbreeding depression was present. The outbreeding depression was observed in the $D_{\text{♀}}R_{\text{♂}}$ hybrid, which had a 27% increase of plasma glucose compared to parental strains. The $D_{\text{♀}}R_{\text{♂}}$ and $R_{\text{♀}}L_{\text{♂}}$ hybrids had more pronounced variations (increase or decrease) in plasma osmolality than their respective parental strains, but these variations were difficult to relate definitively with the potential secondary stress response. These results indicate a strong potential for genetic improvement in the stress response to transport with the use of purebred crosses while hybridization has little value in this regard.

Key words: stress resistance; heterosis; heritability; brook charr

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INTRODUCTION

During aquaculture and stocking activities, fish are faced with several potential stressors. In particular, transportation, but also capture and handling procedures, a highly crowded and confined farming environment, possible air exposure, variation in water quality are all factors that may increase the stress level of organisms (Barton & Iwama, 1991; Iwama *et al.*, 1999; Barton, 2002; Hur *et al.*, 2007). Such stressors may disturb the organism's homeostatic equilibrium, and fish need to compensate by physiological and biochemical changes (Barton & Iwama, 1991; Iwama *et al.*, 1999; Barton, 2002). Three main levels of stress response have been identified (Barton & Iwama, 1991; Iwama *et al.*, 1999; Barton, 2002). The primary neuroendocrine response involves the release of stress hormones—catecholamines and cortisol—into the blood. Biochemical and physiological secondary responses associated with the release of stress hormones activate metabolic pathways that result in the modification of blood chemistry and haematology, including a rapid release of glucose to provide sufficient energy, changes in osmolarity, and lysozyme activity. Finally, tertiary whole-organism and population responses are characterized by changes in the energy supply to the different biological pathways and in population productivity, resulting in negative impacts on growth rate, reproductive success, disease and parasite resistance, saltwater tolerance, and survival (Barton & Iwama, 1991; Fevolden *et al.*, 1991; Pickering, 1993; Barton, 2002; Davis, 2006; Liebert & Schreck, 2006). Therefore, fish with reduced stress response may have an advantage in farming conditions compared to more stress-prone individuals (Fevolden *et al.*, 1991; Fevolden *et al.*, 1993; Pickering, 1993).

73 Differences in the intensity of the stress response have been reported among families and strains of
74 rainbow trout (*Oncorhynchus mykiss* Walbaum) and Atlantic salmon (*Salmo salar* Linnaeus), among
75 strains of fighting fish (*Betta splendens* Regan), and among species of tilapia (*Oreochromis* spp.),
76 guppy (*Poeciliopsis* spp.), and charr (*Salvelinus* spp.) (Bulger & Schultz, 1982; Fevolden *et al.*, 1991;
77 McDonald *et al.*, 1993; Pottinger & Moran, 1993; Cnaani *et al.*, 2004; Verbeek *et al.*, 2008). For
78 example, brook charr (*Salvelinus fontinalis* Mitchill) are less sensitive to transport and net confinement
79 stress (reduced ion loss) compared to lake trout (*Salvelinus namaycush* Walbaum) (McDonald *et al.*,
80 1993). Furthermore, quantitative genetic studies have revealed a moderate to high degree of heritability
81 of the cortisol response for different fishes including carp (*Cyprinus carpio* Linnaeus, 0.60, Tanck *et*
82 *al.*, 2001) and rainbow trout (*O. mykiss*, 0.56 for North American lines, Weber *et al.*, 2008; 0.50 for
83 European lines, Fevolden *et al.*, 2002). Given such additive genetic components, stress resistance—and
84 more specifically variation in stress-induced cortisol concentration—has been identified as a trait of
85 interest for genetic improvement (Fevolden *et al.*, 1991; Lankford & Weber, 2006). However, studies
86 using selective breeding programs for disease resistance or growth that aim to improve fish
87 performance via a lower cortisol response have met with limited success thus far (Lankford & Weber,
88 2006; Weber & Silverstein, 2007).

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91 Another approach that can be considered for the genetic improvement of physiological traits is the
92 production of hybrid crosses that may result in heterosis (i.e., hybrid vigour), which is the improved
93 performance of first generation progeny compared to parental lines (Falconer & Mackay, 1996).
94 Heterosis is the most important non-additive effect on cross performance and is usually stronger when
95 parental lines are genetically distant from each other (Shikano *et al.*, 2000; Wang & Xia, 2002). This
96 phenomenon is now being used in improvement schemes concerning traits of interest in aquaculture,
97 including growth rate, survival, and salinity tolerance (Bentsen *et al.*, 1998; Shikano & Taniguchi,

98 2002; Bryden *et al.*, 2004; Hena *et al.*, 2005). Until now, very few studies have investigated the
99 importance of heterosis on stress response in fish (Campbell *et al.*, 1998; Bryden *et al.*, 2004).

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102 The main objective of this study was to test for the occurrence and to quantify the importance of
103 heterosis in the physiological stress response by comparing three pure strains of brook charr
104 (*S. fontinalis*) and their F1 hybrids. More specifically, the effects of stress induced by transportation, a
105 common activity in aquaculture that often results in mortality, were investigated. A second objective
106 was to estimate heritability values for primary (plasma cortisol) and secondary (plasma glucose, plasma
107 osmolality, and haematocrit) stress indicators for the first time in brook charr and to compare the
108 observed values with other fishes. In this way, the present study planned to evaluate the relative merits
109 of hybrid crosses and selective breeding for improving the response of brook charr to stress in an
110 aquaculture context.

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MATERIALS AND METHODS

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BROOK CHARR STRAINS

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119 Three genetically distinct strains of brook charr (Martin *et al.*, 1997) were used as parental lines.
120 The Laval strain originates from a wild population of anadromous brook charr from the Laval River
121 (48°44'N; 69°05'W) on the north shore of the St. Lawrence Estuary (QC, Canada). The fish used were
122 third generation breeders reared in captivity at the Station aquicole ISMER/UQAR (Rimouski, QC,

123 Canada). The Rupert strain originates from a freshwater-resident wild population inhabiting the Rupert
124 River system (51°05'N; 73°41'W) (QC, Canada). The fish used as breeders were also from the third
125 generation produced in captivity at the Laboratoire régional en sciences aquatiques (LARSA,
126 Université Laval, Québec, QC, Canada). Finally, the so-called “Domestic” strain is the main one being
127 used by the Québec fish farming industry and it originates from two strains (Nashua and Baldwin).
128 Breeders used in this study were obtained from the Pisciculture de la Jacques Cartier (Cap-Santé, QC,
129 Canada). The two wild strains were selected for breed improvement because adults from these
130 populations exhibit late sexual maturation and large adult size.

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133 BREEDING DESIGN

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136 Hybrid and purebred crosses were made from mid-November to the end of December 2005 at
137 LARSA using eggs and milt obtained from the different fish rearing locations. Three purebred crosses
138 were produced: ♀ domestic × ♂ domestic ($D_{\text{♀}}D_{\text{♂}}$), ♀ Laval × ♂ Laval ($L_{\text{♀}}L_{\text{♂}}$), and ♀ Rupert × ♂
139 Rupert ($R_{\text{♀}}R_{\text{♂}}$). Five hybrid and reciprocal hybrid crosses were also produced: $D_{\text{♀}}R_{\text{♂}}$, $D_{\text{♀}}L_{\text{♂}}$, $L_{\text{♀}}D_{\text{♂}}$,
140 $L_{\text{♀}}R_{\text{♂}}$, and $R_{\text{♀}}L_{\text{♂}}$. It was not possible to obtain the $R_{\text{♀}}D_{\text{♂}}$ cross because of the long time lag in sexual
141 maturation between these two strains (October for the domestic males and December for the Rupert
142 females). All breeders were used only once; their mass and length measurements are presented in
143 Table I. For each cross, 10 full-sib families were obtained through single-pair mating. Milt was used
144 fresh (immediately after collection) without any additive. The numbers of eggs fertilized for each
145 female were not counted and all were incubated. The number of fry per family was equalized after
146 exogenous feeding had begun. Eight of the resulting 80 families were eliminated due to the limited
147 number of individuals that could be pooled in each tank.

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150 FAMILY REARING

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153 From egg incubation (January) to exogenous feeding (June), each family was incubated separately
154 in individual clays, and each incubation tank contained 12 clays. Water temperature was maintained at
155 6°C during egg incubation and at 8°C after hatching. The photoperiod was set at 12L:12D. In June,
156 families were identified using different combinations of adipose and pelvic fin clippings and
157 transferred to nine 3 m³ tanks, with eight families pooled per tank. All families were brought to 2136
158 degrees-days by the end of the summer and maintained at 10°C in recirculating fresh water.
159 Photoperiod followed the natural seasonal cycle, and fish were fed according to commercial charts with
160 commercial pellets. In September, fish were transferred in transport bags (one family per bag)
161 immediately to the Station aquicole ISMER/UQAR. Here they were reared in ten 0.5 m³ indoor tanks,
162 with six to eight families per tank, under natural temperature and photoperiod conditions in running
163 dechlorinated fresh water. Fish were fed daily (1% w/w ration) with commercial dry pellets. No
164 mortality difference was observed among cross types during the whole rearing period. There was no
165 disease occurrence, and prophylactic treatments (chloramines T) were applied following marking and
166 weight and length measurements.

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169 STRESS EXPOSURE

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172 A simulation of fish transfer procedures in transport bags was conducted in June 2007 to induce
173 stress in 16-month-old fish. Twenty fish per cross were used for this experiment. The fish were
174 captured in tanks, taking care that a similar number of fish from the different families within each
175 cross-type were chosen, i.e., 2 to 3 fish per family, and randomly distributed among bags. Each
176 transport bag (30 cm in diameter, 100 cm in length) contained 10 fish that were kept in 1/3
177 dechlorinated fresh water (same water source as the holding tanks) and 2/3 compressed oxygen (16
178 bags with a total of 160 fish). Transportation bags were kept in the dark and shaken every 30 min for
179 10 s. Fish were kept in the bags for 4 h, which is long enough to induce an intense stress response in
180 brook charr (McDonald *et al.*, 1993). After 4 h, the bags were put into fresh water to let the temperature
181 gradually decrease to the tank temperature (about 20 min), and fish were then sampled. Twenty fish per
182 cross were also sampled directly from fish tanks and used as controls. No mortality was observed in
183 transport bags or rearing tanks during the experiment.

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186 SAMPLING PROCEDURES

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189 All samplings were made between 16:00 and 19:00 to avoid bias due to endocrine circadian
190 rhythms. Stressed and control fish were anaesthetized in MS 222 (0.16 g l⁻¹ [3-aminobenzoic acid ethyl
191 ester]) and their body mass (to the nearest 0.1 g) and fork length (0.1 cm) were measured (Tables II and
192 III). Blood was collected by caudal puncture using ammonium-heparinized syringes. A small quantity
193 of blood was transferred to capillary tubes for haematocrit determination and the remainder was
194 centrifuged at 7200g for 3 min. The plasma was drawn off, quickly frozen in liquid nitrogen, and then
195 stored at -80°C until analysis. Plasma osmolality was measured with an Advanced Micro-osmometer

196 3MO, plasma glucose was measured by enzymatic determination (Alexander & Griffiths, 1993), and
197 cortisol levels were measured using a cortisol ¹²⁵I RIA kit (MP Biomedicals, Orangeburg, NY, USA).

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200 STATISTICAL ANALYSES

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202

203 Data normality and homogeneity of variance were tested with Kolmogorov-Smirnov and Brown-
204 Forsythe tests, respectively. Plasma cortisol concentrations were log transformed to obtain normality.
205 The variability between replicate transport bags was tested using ANOVA and was not significant
206 (results not shown). The different variables were analyzed using two-way ANOVAs with cross-type,
207 stress treatment, and stress treatment × cross-type interaction as fixed effects. The effect of dam and
208 sire origin (domestic, Laval, or Rupert) on each physiological variable after stress exposure was
209 analyzed using two-way ANOVAs with dam and sire origin as factors. The presence of heterosis or
210 outbreeding depression was determined by the presence of a significant difference between the mean
211 performance of hybrids compared to the mean performance of both parental strains (Bryden *et al.*,
212 2004). Heterosis was expressed when there was a lower stress response in hybrids compared to parental
213 lines. *A posteriori* Tukey tests were used for mean comparisons when possible or replaced by Games
214 and Howell tests when variances were not homogenous. The influence of fish mass on variables was
215 examined using mass as a covariate in ANCOVAs. Analyses were made using Statistica version 6.0 for
216 Windows (StatSoft, Tulsa, OK, USA). A significance level of $\alpha = 0.05$ was used in all statistical tests.

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219 HERITABILITY ANALYSES

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222 Our breeding design was used to fit animal models (Lynch & Walsh, 1998) with the ASReml
223 software (V2.0; Gilmour *et al.*, 2006). Univariate analyses were used to decompose the phenotypic
224 variance (V_P) of each trait for the whole fish population (including pure and hybrid crosses) into their
225 additive genetic (V_A) and residual (V_R) variances. The model was the following:

226

$$y = \mu + C + A + e$$

227 where y is the phenotypic observation, μ is the overall mean, C is the fixed effect of the cross-type, A is
228 the random additive genetic effect, and e is the random residual effect. The narrow-sense heritability
229 (h^2) for each trait was estimated as the ratio of the additive genetic variance (V_A) to the total phenotypic
230 variance (V_P): $h^2 = V_A/V_P$. The statistical significance of the additive genetic component for each trait
231 was tested by re-running a restricted model where the additive variance was set to zero and then
232 comparing the difference the in log-likelihood ratio between the original and the restricted model
233 against the chi-square distribution ($df = 1$), where $\chi^2 = -2 * \text{difference in log likelihood}$.

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RESULTS

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239 PLASMA CORTISOL RESPONSE

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242 A stress response was noted in every cross-type, as shown by a significant increase in cortisol
243 between control and stressed fish (Table IV; Fig. 1). However, the intensity of the cortisol response
244 was variable depending on the cross, with significant interactions observed between stress treatment
245 and cross-types (Table IV; Fig. 1). All control fish had the same level of initial plasma cortisol (Fig. 1).

246 The stress treatment in purebred crosses induced a significantly lower cortisol response in the Rupert
247 fish than in Laval and domestic fish, with the last two being similar (Fig. 1A). In hybrids, when the
248 Rupert strain was used as either dam or sire, the post-stress cortisol level did not differ significantly
249 from either parental line (Fig. 1B; 1D). In crosses involving the domestic and the Laval strains, all
250 hybrids and parental lines showed similar cortisol responses (Fig. 1C). These results are indicative of
251 an additive response rather than a non-additive effect. Mass had no significant effect on this trait (Table
252 IV).

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255 SECONDARY STRESS RESPONSE INDICATORS

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258 A significant interaction was observed between stress treatment and cross-type for glucose
259 concentration (Table IV). Plasma glucose concentrations were similar for all controls (Fig. 2) while
260 they were significantly higher after stress exposure in all cross-types (Table IV; Fig. 2). The glucose
261 response was similar among the three purebred lines (Fig. 2A), and hybrids showed concentrations
262 similar to their parental lines (Fig. 2C; 2D). The only exception was the D♀R♂ hybrid, which had a
263 significantly higher glucose concentration after stress exposure (Fig. 2B), hence expressing outbreeding
264 depression. Glucose concentration was 27% higher in this hybrid after stress exposure compared to the
265 average glucose concentration in parental lines. There was no significant co-factor effect for mass
266 (Table IV).

267

268

269 A significant interaction was observed between stress treatment and cross-type in the plasma
270 osmolality response to transport stress (Table IV; Fig. 3). In purebred lines, controls were not different

271 (Fig. 3A). Following stress exposure, Laval fish had significantly higher plasma osmolality levels than
272 controls while osmolality did not vary in the other two purebred lines (Fig. 3A). Pre-stress levels of
273 plasma osmolality were similar to both parental lines in the D♀R♂ and D♀L♂ hybrids (Fig. 3B and 3C),
274 similar to the Laval line in the L♀D♂ hybrid (Fig. 3C), and similar to the Rupert line in hybrids between
275 the Rupert and the Laval lines (Fig. 3D). After stress exposure, there was a significant increase in
276 plasma osmolality in the D♀R♂ hybrid while no change was observed in the parental lines (Fig. 3B).
277 The reverse was observed in the R♀L♂ hybrid, with a significant decrease in plasma osmolality (Fig.
278 3D). As with the Rupert line, no osmolality change was observed in the L♀R♂ hybrids (Fig. 3D), and
279 hybrids between the domestic and the Laval strains behaved in a way similar to their maternal strain
280 (Fig. 3C). The interaction between stress treatment and cross-type was significant for the blood
281 haematocrit response (Table IV). Blood haematocrit was similar among controls and increased only in
282 the domestic line after stress exposure (Fig. 4). For both plasma osmolality and blood haematocrit, the
283 mass co-factor was significant (Table IV) but correlations were weak ($r = 0.15$ for both).

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286 HERITABILITY

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289 Significant additive genetic variance and heritability were obtained at both stress response levels for
290 the whole population. Heritability estimates for cortisol ($h^2 = 0.60 \pm 0.20$) and glucose ($h^2 = 0.61 \pm$
291 0.20) following stress exposure were high and significant (Table V), while estimates were not
292 significant for osmolality ($h^2 = 0 \pm 0$) or haematocrit ($h^2 = 0.46 \pm 0.25$) (Table V).

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295 PARENTAL ORIGIN EFFECTS

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298 Dam and sire origin significantly affected the stress response depending on the trait as was the case
299 for heritability, the parental origin effect was strong for cortisol (Table VI). However, the results for the
300 secondary response show different tendencies (~~Table VI~~): (i) there were significant effects of both dam
301 and sire origin in the cortisol response, with fish issued from the Rupert strain having lower plasma
302 cortisol than other fish (~~Table VI~~); (ii) no significant dam or sire effect was observed for the glucose
303 response (~~Table VI~~); and (iii) there was a significant dam origin effect on the osmolality and
304 haematocrit stress responses (Table VI). Progeny of Rupert dams had lower plasma osmolality
305 following stress exposure than progeny of the other two strains when used as dams, and progeny of
306 Laval dams had lower haematocrit after stress exposure than when domestic dams were used.

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DISCUSSION

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312 Our main objectives were to determine whether heterosis occurred and to estimate the heritability of
313 primary and secondary stress indicators in brook charr (*S. fontinalis*). While our results revealed no
314 clear evidence of heterosis, relatively high heritability was found for endocrine and physiological
315 responses. A third objective was to compare the stress response between strains of brook charr. Inter-
316 strain differences have been previously reported between unselected lines of fighting fish (*B.*
317 *splendens*) and also between lines selected for different response to stress in rainbow trout (*O. mykiss*)
318 and Atlantic salmon (*S. salar*) (Fevolden *et al.*, 1991; Pottinger, 2006; Verbeek *et al.*, 2008). In these
319 studies, the stress cortisol response varied by 1.25 to 2 times when the most sensitive population is

320 compared to the least sensitive one. Our results indicate a similar range, with the Rupert strain response
321 being about half those of the other purebred strains.

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323

324 PUREBRED LINES

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327 As previously indicated based on the primary and secondary stress responses, the Rupert strain
328 displayed a less pronounced response to transport stress while the Laval strain seemed to be the most
329 sensitive. The osmoregulatory disturbance in the Laval strain is not easy to interpret since a secondary
330 stress response would have resulted in decreased osmolality in a freshwater fish. The domestic strain
331 was the only one to show an increase in haematocrit, which may reflect a need for oxygen to
332 compensate stress (Casillas & Smith, 1977). Some studies have revealed an impact of growth selection
333 on stress performance, with a greater response to stress challenge and a longer stress recovery in
334 heavier fish (Casillas & Smith, 1977; Lankford & Weber, 2006; Weber & Silverstein, 2007), while
335 others observed no such correlation (Fevolden *et al.*, 1991; Millot *et al.*, 2009). Here, only weak
336 correlations were present between mass and either the primary or secondary stress responses, indicating
337 a weak link and therefore limited effect of body mass on stress resistance in brook charr.

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340 NON-ADDITIVE GENETIC EFFECTS

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343 No non-additive components seemed to influence the cortisol response; this is similar to findings on
344 other species (channel catfish, *Ictalurus punctatus* [Rafinesque], Bosworth *et al.*, 2004; Chinook

345 salmon, *Oncorhynchus tshawytscha* [Walbaum], Bryden *et al.*, 2004). Studies on hybrids have rarely
346 provided evidence of non-additive effects, but they generally focussed on survival or cortisol response
347 (Bulger & Schultz, 1982; Bosworth *et al.*, 2004; Bryden *et al.*, 2004). However, heterosis related to
348 survival time (tertiary response) was reported in F1 hybrids after salinity stress in *Poecilia reticulata*
349 Peters (Chiyokubo *et al.*, 1998) and heat stress in *Poeciliopsis monacha-occidentalis* Angus (Bulger &
350 Schultz, 1982).

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352

353 A weak but significant non-additive component was present at the physiological level (secondary
354 response), especially for plasma glucose concentration in the D♀R♂ hybrid. A non-additive component
355 was also observed for plasma osmolality in D♀R♂ and R♀L♂ hybrids, but this is more difficult to
356 interpret for the D♀R♂ hybrid, as previously mentioned. Our observations of non-additive components
357 only at the secondary response level reveal the presence of genetic divergence in purebred strains at the
358 physiological level rather than a neuroendocrine response to stress stimuli. The extents of non-additive
359 genetic phenomena are thought to be principally linked to the genetic distance between parental lines
360 (Falconer & Mackay, 1996; Tymchuk *et al.*, 2007). If the lines are too genetically distant or adapted to
361 their own environment, hybrids can show outbreeding depression with a breakdown of genetic complex
362 associations; on the other hand, when the genetic distance between parental strains is closer, hybrids
363 can express heterosis (Falconer & Mackay, 1996; Shikano *et al.*, 2000; Cooke *et al.*, 2001). Our results
364 do not support any of these expectations according to genetic distance: (1) the Laval and Rupert strains
365 were the most genetically distant strains (Martin *et al.*, 1997), and one of their hybrids (R♀L♂)
366 expressed a response significantly different from the parental responses for osmolality; and (2) the
367 D♀R♂ hybrid expressed outbreeding depression (glucose) while the two parental lines were more
368 genetically similar. In addition, the results obtained for the other hybrids do not support the hypothesis
369 that the genetic distance would be the main effect involved in non-additive expression in our crosses.

370 Other effects related to genetic architecture (e.g., epistasis, pleiotropy, or genetic linkage) should be
371 explored to explain our results. Overall, the presence of non-additive genetic effects only in secondary
372 stress responses suggests that the use of hybrids to improve transport stress resistance in aquaculture
373 has limited potential.

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376 ADDITIVE GENETIC EFFECTS

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379 The primary response to stress, i.e., cortisol response, seems to be principally under additive genetic
380 control. The plasma cortisol concentration in hybrids was always similar to both parental lines. Both
381 dam and sire origin significantly affected this trait, indicating the importance of an additive genetic
382 basis underlying this stress response. Other studies on hybrids also revealed additive effects on plasma
383 cortisol level after exposure to stress: Bryden *et al.* (2004) exposed wild and farm Chinook salmon (*O.*
384 *tshawytscha*) hybrids and purebred crosses to an “aerial emersion” stressor, and the cortisol response in
385 hybrids was equal to both parental lines. The high additive component for cortisol regulation translated
386 into high and significant heritability estimate for this trait ($h^2 = 0.60 \pm 0.20$). The cortisol response to
387 stress is already used for genetic improvement in other fish species, especially in rainbow trout (*O.*
388 *mykiss*), in which heritability values similar to those obtained in our study have been documented in the
389 F1 generation (h^2 ranging from 0.41 to 0.56 depending on strain origin) (Pottinger & Carrick, 1999;
390 Fevolden *et al.*, 2002; Overli *et al.*, 2005; Weber & Silverstein, 2007; Weber *et al.*, 2008). The
391 selection procedure for stress response in rainbow trout was based on the mean post-stress plasma
392 cortisol response across five episodes of confinement stress testing on parental lines, with the highest
393 responding (HR) or lowest responding (LR) individuals used to produce the next generation. This
394 breeding program was repeated several times to obtain F2 and F3 generations with improved stress

395 resistance and other possibly related traits, such as increased growth or disease resistance (Pottinger &
396 Carrick, 1999; Overli *et al.*, 2005; Ruiz-Gomez *et al.*, 2008). Our results suggest that such a program
397 could also be applied in brook charr.

398

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400 For the secondary stress response, plasma glucose concentration also displayed significant
401 heritability estimates. This trait had higher heritability ($h^2 = 0.61 \pm 0.20$) than values reported in
402 previous studies on androgenetic carp (*C. carpio*, 0.19; Tanck *et al.*, 2001), Atlantic salmon (*S. salar*,
403 0.03; Fevolden *et al.*, 1993), and rainbow trout (*O. mykiss*, 0.07; Fevolden *et al.*, 1993). The low
404 heritability observed in carp could be related to the androgenetic design, i.e., the UV irradiation and
405 heat shock treatment might induce additional environmental variation due to embryonic damage caused
406 by the androgenetic shock treatment and therefore reduce heritability (Tanck *et al.*, 2001). On the other
407 hand, our own estimates could have been inflated due to our full-sib design, which may include other
408 sources of variance including maternal effects (Falconer & Mackay, 1996; Pante *et al.*, 2002).
409 However, previous studies in brook charr revealed that while maternal effects are apparent during the
410 very first stages of development, they vanish within several months following hatching (Perry *et al.*,
411 2004; Perry *et al.*, 2005). This suggests that maternal effect should have a limited impact on our results.
412 No significant heritability was found for osmolality or haematocrit response. Until now, no study has
413 documented the heritability of osmolality variations related to stress resistance, but a very low
414 heritability for haematocrit was reported in clonal lines of ayu (*Plecoglossus altivelis* [Temminck &
415 Schlegel], 0.072; DelValle *et al.*, 1996).

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418 In summary, the significant heritability of stress response at both the primary (cortisol) and
419 secondary (glucose) levels indicates a good potential for selective breeding and genetic improved

420 resistance to transport stress in brook charr, and particularly so for the Rupert strain. Future work
421 should aim at determining whether the difference expressed among strains is the result of global stress
422 sensitivity variations or if some strains are more sensitive than others to different types of stress. On the
423 opposite, hybridization does not seem to be a promising avenue to improve stress resistance in brook
424 charr. Nevertheless, it would be worth further investigating this issue by comparing strains specifically
425 selected for different sensitivity to stress response which was not the case here. Thus, fixation of alleles
426 related to the stress response in different strains could produce different, non-additive physiological
427 effects in mixed progenies.

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432

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562 059.1

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564

1 Table I: Total mass (Kg) and length (cm) of the breeders used to produce the different purebred (bold)
 2 and hybrid cross-types. Mean \pm SE; n is the number of individuals; different letters indicate significant
 3 differences among cross-types ($\alpha = 0.05$).

Cross	Female			Male		
	n	mass	length	n	mass	length
D ♀ R ♂	10	0.59 \pm 0.02 ^{ab}	35.72 \pm 0.40 ^a	10	0.63 \pm 0.04 ^a	37.72 \pm 1.02 ^a
D♀D♂	10	0.70 \pm 0.02^c	36.75 \pm 0.36^a	10	0.81 \pm 0.03^a	38.42 \pm 0.70^a
D ♀ L ♂	10	0.78 \pm 0.07 ^{bcd}	38.05 \pm 1.30 ^{ab}	10	1.03 \pm 0.12 ^{ab}	43.95 \pm 0.66 ^{bc}
L ♀ D ♂	10	0.97 \pm 0.10 ^{cd}	41.25 \pm 0.73 ^b	10	0.71 \pm 0.03 ^a	37.68 \pm 0.42 ^a
L♀L♂	10	1.07 \pm 0.08^d	42.60 \pm 0.87^b	10	1.25 \pm 0.06^{bc}	44.83 \pm 0.63^{bc}
L ♀ R ♂	10	1.16 \pm 0.14 ^c	42.21 \pm 0.74 ^b	10	0.85 \pm 0.09 ^{ab}	40.26 \pm 1.27 ^{ab}
R ♀ L ♂	10	1.39 \pm 0.21 ^{bcd}	45.46 \pm 2.01 ^b	10	1.46 \pm 0.17 ^c	46.34 \pm 0.62 ^a
R♀R♂	10	0.47 \pm 0.04^a	35.71 \pm 1.01^a	10	0.77 \pm 0.11^a	40.33 \pm 1.75^{abc}

4

5

6 Table II: Total mass (g) and length (cm) of the three purebred strains (**bold**) and their hybrids used as
7 controls or for the stress challenge. Mean \pm SE; n is the number of individuals; different letters indicate
8 significant differences among cross-types ($\alpha = 0.05$).

Cross	n	Control		n	Stressed	
		mass	length		mass	length
D♀R♂	20	41.87 \pm 2.07 ^{de}	15.69 \pm 0.25 ^c	20	49.26 \pm 4.16 ^{de}	16.53 \pm 0.43 ^c
D♀D♂	20	58.24 \pm 5.48^e	16.63 \pm 0.49^c	20	61.53 \pm 5.25^e	17.25 \pm 0.49^c
D♀L♂	20	37.82 \pm 3.47 ^{cd}	15.02 \pm 0.45 ^{bc}	20	39.12 \pm 4.02 ^{cd}	15.38 \pm 0.45 ^{bc}
L♀D♂	20	33.36 \pm 2.39 ^{cd}	14.73 \pm 0.34 ^{bc}	20	45.39 \pm 4.05 ^{cd}	16.41 \pm 0.41 ^c
L♀L♂	26	15.59 \pm 1.01^a	11.94 \pm 0.29^a	30	14.03 \pm 0.70^a	11.49 \pm 0.18^a
L♀R♂	20	24.48 \pm 2.09 ^{bc}	13.56 \pm 0.39 ^{ab}	20	31.85 \pm 3.23 ^{bc}	14.93 \pm 0.53 ^{bc}
R♀L♂	20	23.91 \pm 2.25 ^b	13.53 \pm 0.36 ^{ab}	20	21.27 \pm 1.72 ^b	13.13 \pm 0.33 ^{ab}
R♀R♂	21	22.75 \pm 1.50^b	13.20 \pm 0.28^{ab}	20	22.42 \pm 1.48^b	13.23 \pm 0.28^b

9 Table III: Summary of two-way ANOVAs for body mass and length. df is degrees of freedom; MS is
 10 mean square; F is the F-ratio.

	Mass (g)				Length (cm)			
	df	MS	F	<i>P</i> -value	df	MS	F	<i>P</i> -value
Stress treatment	1	633.0	1.6	> 0.1	1	21.4	7.3	< 0.01
Cross-type	7	9455.7	49.9	< 0.001	7	137.2	46.6	< 0.001
Stress treatment × Cross-type	7	278.4	1.5	> 0.1	7	6.5	2.2	< 0.05
Error	321	189.4			321	2.9		
Model R ²	0.53				0.52			
Adjusted R ²	0.51				0.50			

11

12 Table IV: Summary of two-way ANOVAs for cortisol, glucose, osmolality, and haematocrit. df is degrees of freedom; MS is mean square; F is the F-
 13 ratio.

	Cortisol (ng ml ⁻¹)				Glucose (mg ml ⁻¹)				Osmolality (mosm kg ⁻¹)				Haematocrit (%)			
	df	MS	F	<i>P</i> -value	df	MS	F	<i>P</i> -value	df	MS	F	<i>P</i> -value	df	MS	F	<i>P</i> -value
Mass (co-variable)	1	0.2	1.7	> 0.1	1	0.2	2.2	> 0.1	1	468.0	8.7	< 0.01	1	0.03	7.9	< 0.01
Stress treatment	1	108.6	1132.0	< 0.001	1	28.4	410.8	< 0.001	1	127.0	2.4	> 0.1	1	0.02	6.4	< 0.01
Cross-type	7	0.2	2.2	< 0.05	7	0.3	4.2	< 0.001	7	303.0	5.6	< 0.001	7	0.01	1.9	> 0.05
Stress treatment × Cross-type	7	0.2	2.2	< 0.05	7	0.2	2.3	< 0.05	7	431.0	8.0	< 0.001	7	0.01	2.3	< 0.05
Error	300	0.1			289	0.1			274	54.0			278	0.01		
Model R ²	0.80				0.62				0.29				0.14			
Adjusted R ²	0.79				0.60				0.25				0.09			

14

15 Table V: Genetic components of the different traits in the stress responses. Estimates of total
 16 phenotypic (V_P), additive (V_A), and residual (V_R) variance components and heritability (h^2) with their
 17 standard errors (\pm SE); n is the number of individuals. *P*-values were obtained from a likelihood ratio
 18 test.
 19

	n	V_P	V_R	V_A	h^2	<i>P</i> -value
Cortisol	159	0.14 \pm 0.03	0.06 \pm 0.02	0.08 \pm 0.04	0.60 \pm 0.20	< 0.05
Glucose	158	0.17 \pm 0.04	0.07 \pm 0.02	0.11 \pm 0.06	0.61 \pm 0.20	< 0.05
Osmolality	148	58.92 \pm 7.04	58.92 \pm 7.04	0	0	> 0.1
Haematocrit	146	0.004 \pm 0.001	0.002 \pm 0.001	0.002 \pm 0.002	0.46 \pm 0.25	> 0.1

20 Table VI: Dam and sire origin effects on the different traits after stress exposure. Physiological traits are expressed as mean \pm SE. Different letters
 21 indicate significant differences among cross-types ($\alpha = 0.05$); P -value indicates the significance level.

	Dam				Sire			
	Domestic	Laval	Rupert	P -value	Domestic	Laval	Rupert	P -value
Cortisol (ng ml ⁻¹)	46.39 \pm 4.85 ^b	47.06 \pm 4.60 ^b	28.96 \pm 3.07 ^a	< 0.05	53.19 \pm 5.85 ^b	44.78 \pm 3.78 ^b	32.78 \pm 4.40 ^a	< 0.01
Glucose (mg ml ⁻¹)	1.33 \pm 0.04	1.24 \pm 0.05	1.19 \pm 0.05	> 0.05	1.23 \pm 0.05	1.21 \pm 0.04	1.34 \pm 0.05	> 0.05
Osmolality (mosm kg ⁻¹)	310.42 \pm 0.94 ^b	309.05 \pm 1.14 ^b	303.52 \pm 1.47 ^a	< 0.01	307.87 \pm 1.25	307.57 \pm 0.96	309.85 \pm 1.33	> 0.05
Haematocrit (%)	0.40 \pm 0.01 ^b	0.37 \pm 0.01 ^a	0.37 \pm 0.01 ^{ab}	< 0.01	0.39 \pm 0.01	0.38 \pm 0.01	0.37 \pm 0.01	> 0.1

22

1 Figure Captions

2

3 Fig. 1: Cortisol (ng ml^{-1}) stress response in the three purebred strains (A) and hybrids between (B)
4 domestic and Rupert strains, (C) domestic and Laval strains, and (D) Laval and Rupert strains. The first
5 letter of the cross-type indicates the dam and the second letter the sire. Open bars are for controls and
6 solid bars for stressed. Statistical analyses were made on log-transformed data but results are presented
7 as mean \pm SE. Different letters indicate significantly different means ($\alpha = 0.05$).

8

9 Fig. 2: Plasma glucose (mg ml^{-1}) stress response in the three purebred strains (A) and hybrids between
10 (B) domestic and Rupert strains, (C) domestic and Laval strains, and (D) Laval and Rupert strains. The
11 first letter of the cross-type indicates the dam and the second letter the sire. Open bars are for controls
12 and solid bars for stressed. Mean \pm SE. Different letters indicate significantly different means ($\alpha =$
13 0.05).

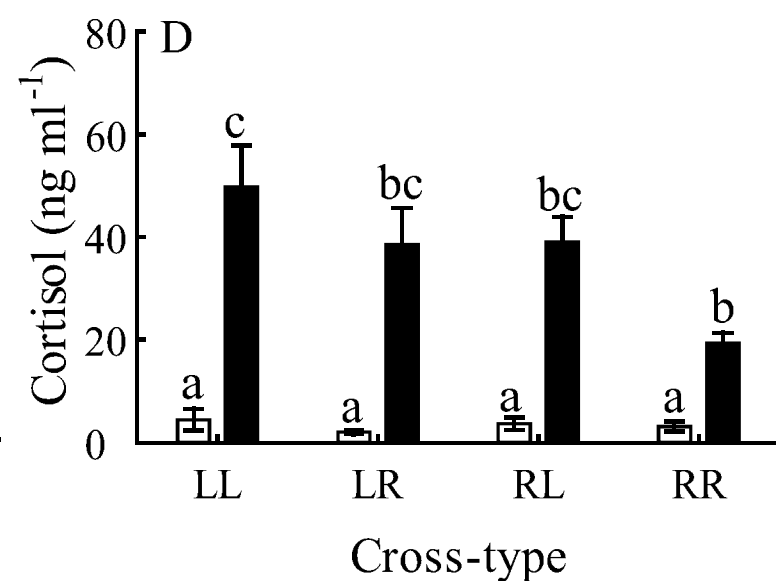
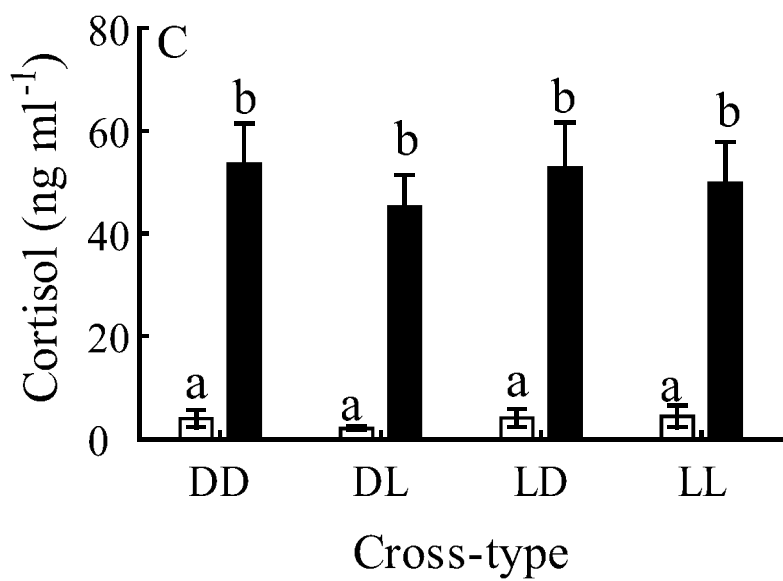
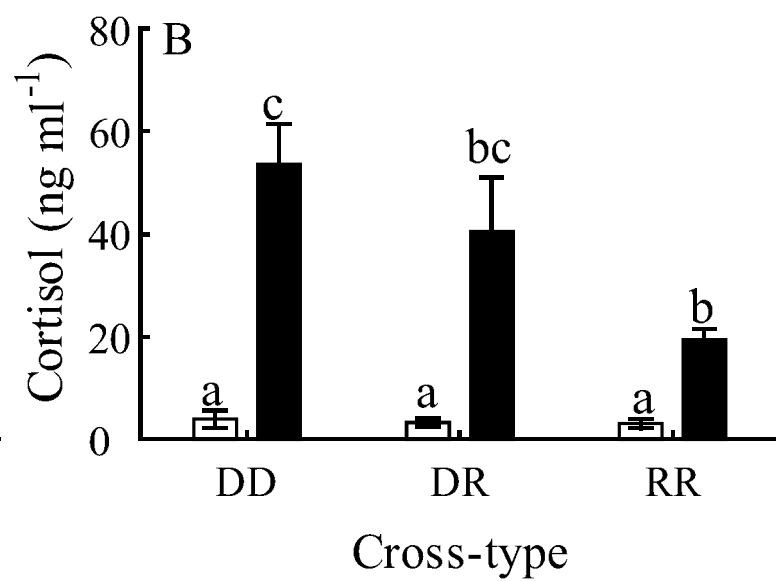
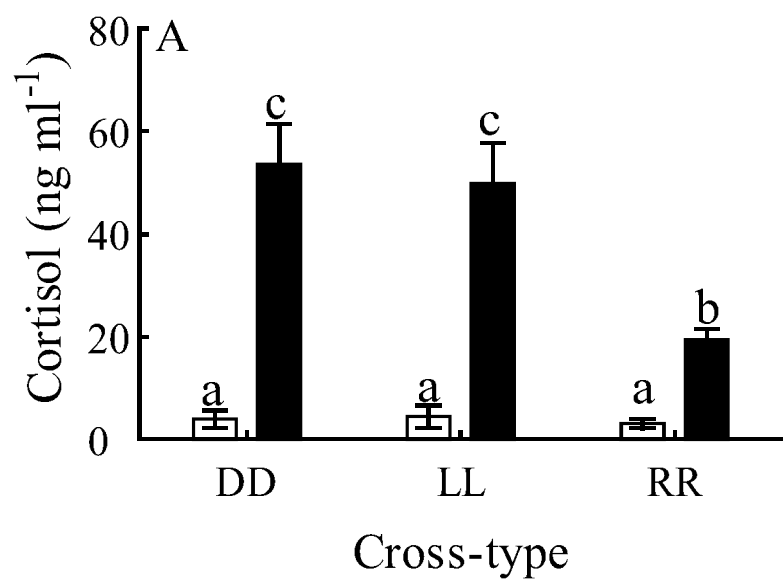
14

15 Fig. 3: Osmolality (mosm kg^{-1}) stress response in the three purebred strains (A) and hybrids between
16 (B) domestic and Rupert strains, (C) domestic and Laval strains, and (D) Laval and Rupert strains. The
17 first letter of the cross-type indicates the dam and the second letter the sire. Open bars are for controls
18 and solid bars for stressed. Mean \pm SE. Different letters indicate significantly different means among
19 controls and asterisks indicate significantly different means between control and stressed ($\alpha = 0.05$).

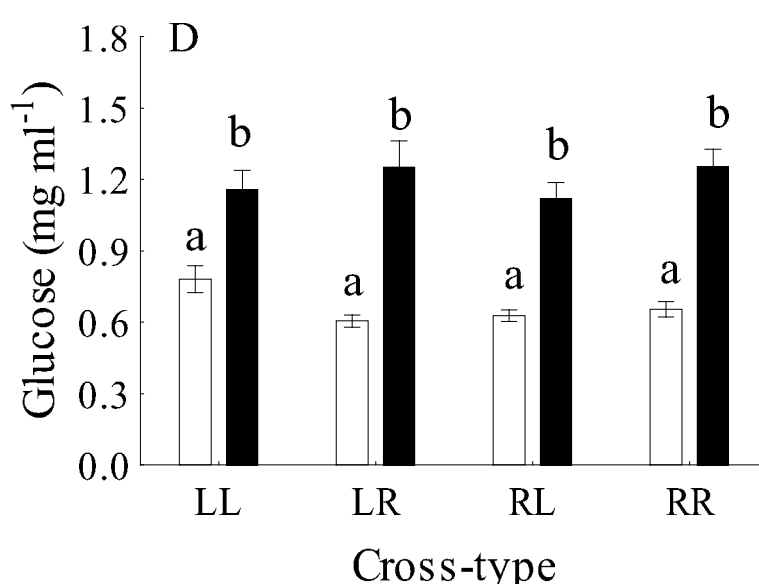
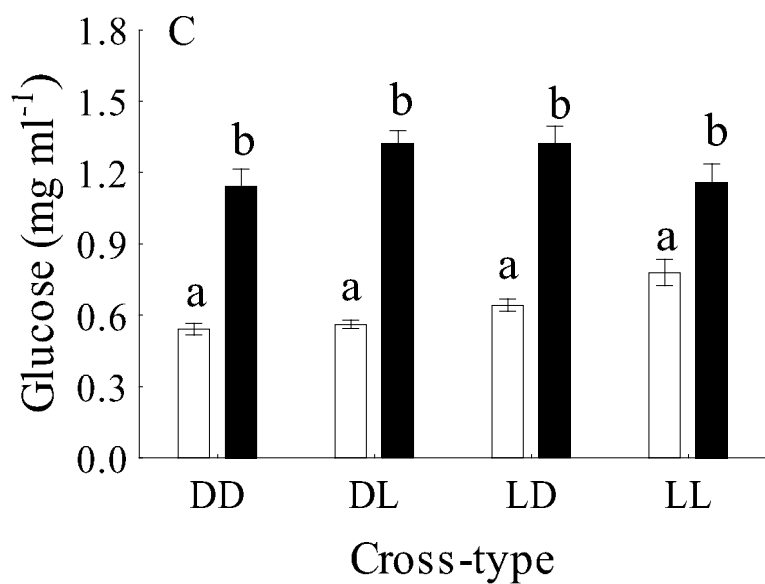
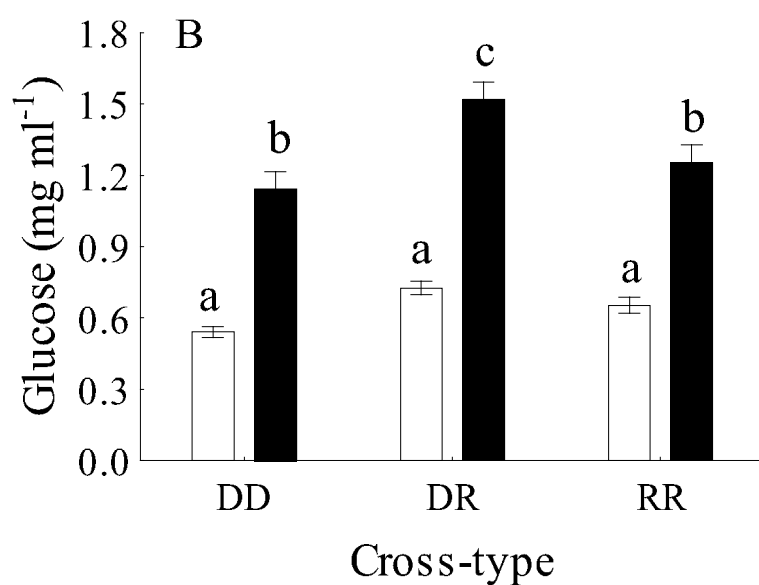
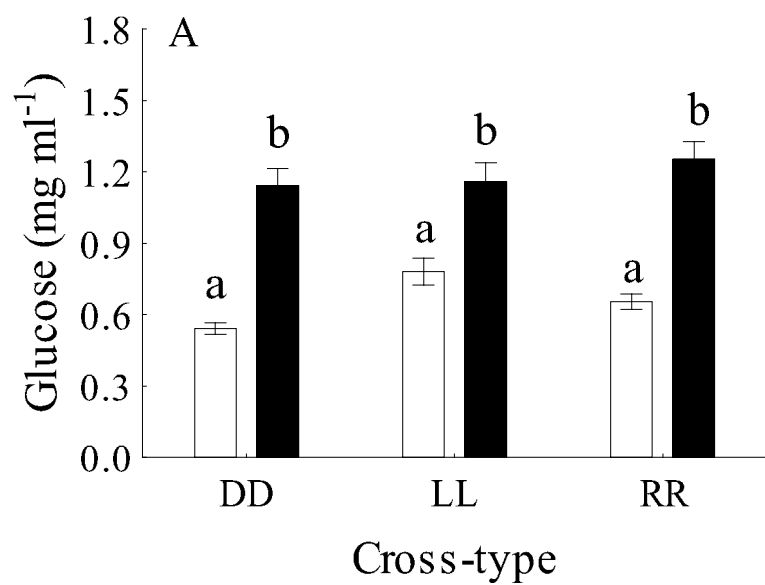
20

21 Fig. 4: Haematocrit (%) stress response in the three purebred strains and their hybrids. The first letter of
22 the cross-type indicates the dam and the second letter the sire. Open bars are for controls and solid bars
23 for stressed. Mean \pm SE. Asterisks indicate significantly different means between control and stressed
24 ($\alpha = 0.05$).

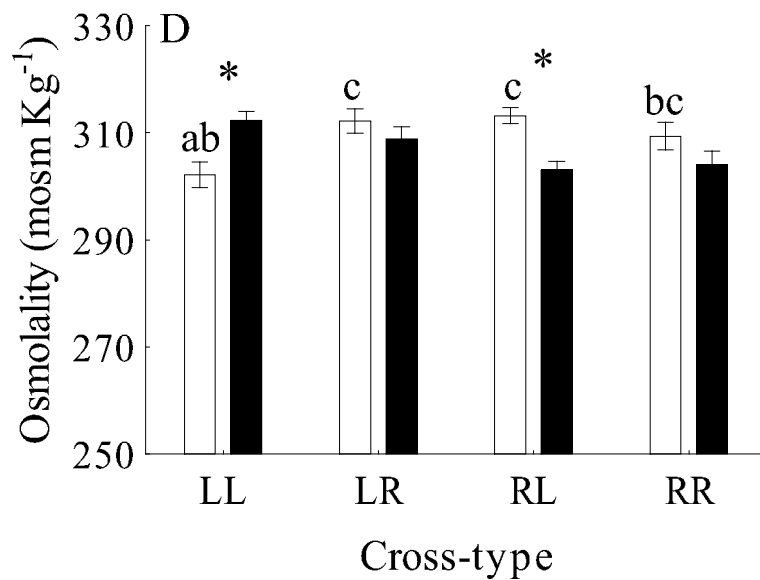
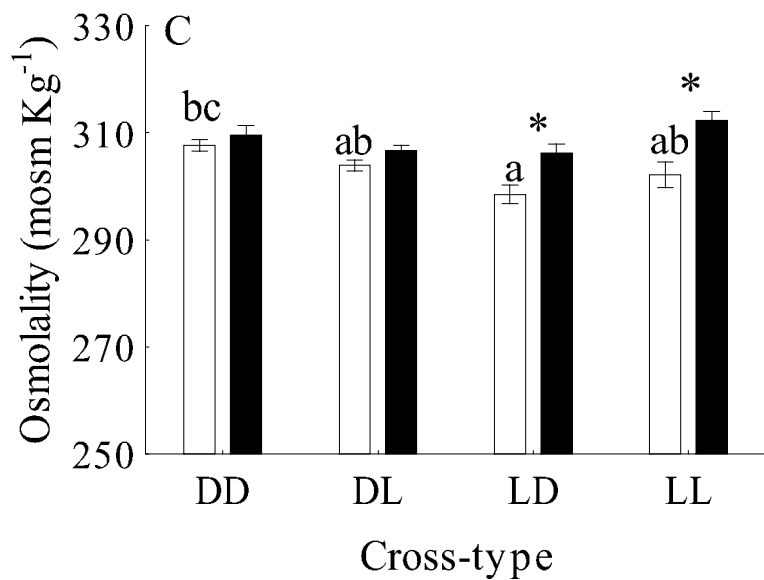
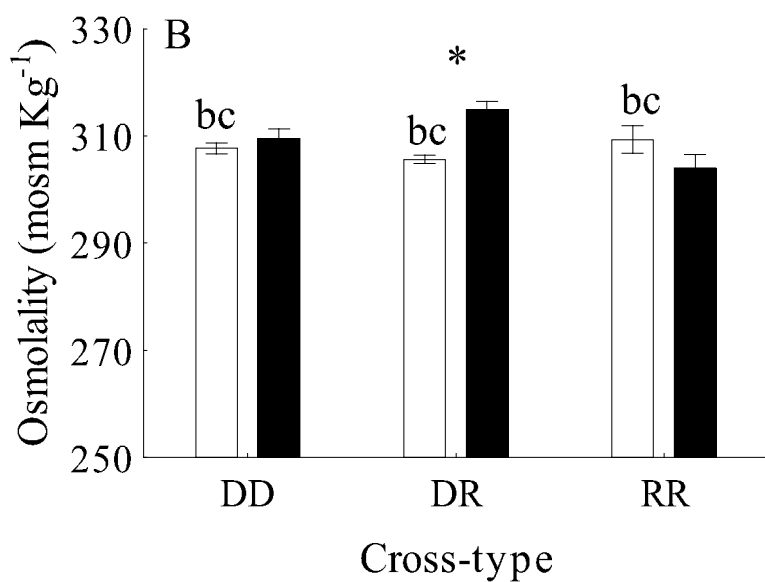
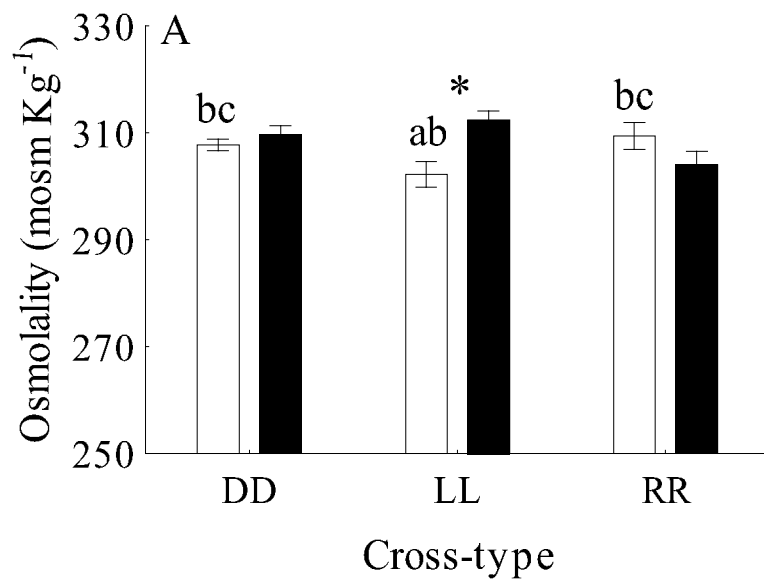
Figure



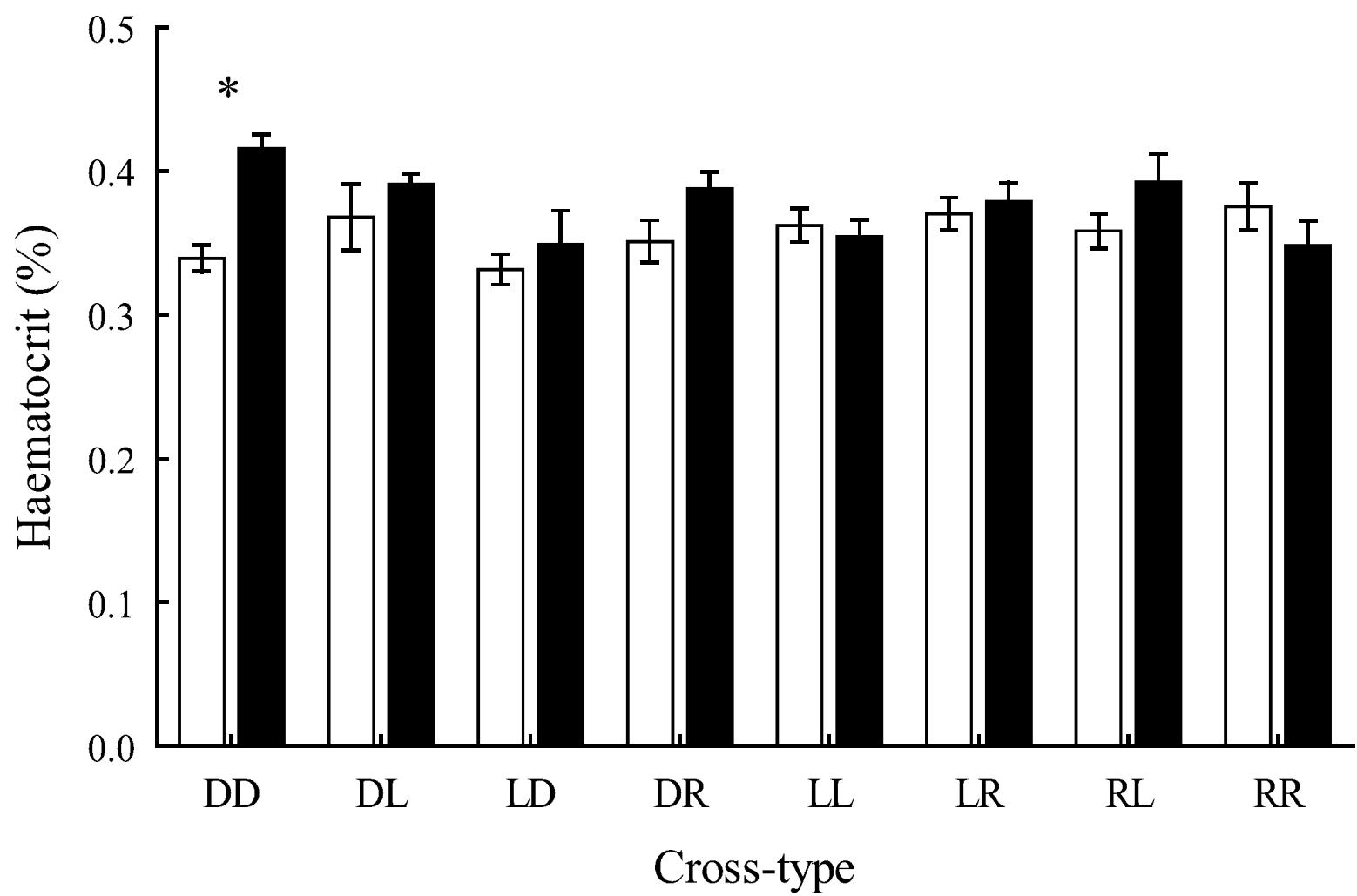
Figure



Figure



Figure



This piece of the submission is being sent via mail.