Quantitative genetic analysis of the physiological stress response in three strains of brook charr *Salvelinus fontinalis* and their hybrids

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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 mean $\pm$ s.e. heritability $(h^2) = 0.60 \pm 0.20$ for plasma cortisol and 0.61 ± 0.20 for plasma glucose. There were strain differences whereby the R 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 $D_{\female}R_{\male}$ hybrid, which had a 27% increase of plasma glucose compared to parental strains. The $D_{\female}R_{\male}$ and $R_{\female}L_{\male}$ 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. © 2011 The Authors

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INTRODUCTION

During aquaculture and stocking activities, fishes are faced with several potential stressors. In particular, transportation, capture and handling procedures, a highly crowded and confined farming environment, possible air exposure and 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 fishes need to compensate by physiological and biochemical changes (Barton & Iwama, 1991; Iwama *et al*.,

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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 effects 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, fishes with reduced stress response may have an advantage in farming conditions compared to more stress-prone individuals (Fevolden *et al.*, 1991, 1993; Pickering, 1993).

Differences in the intensity of the stress response have been reported among families and strains of rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) and Atlantic salmon *Salmo salar* L. 1758, among strains of fighting fish *Betta splendens* Regan 1910 and among species of tilapia (*Oreochromis* spp.), guppy (*Poeciliopsis* spp.) and charr (*Salvelinus* spp.) (Bulger & Schultz, 1982; Fevolden *et al.*, 1991; McDonald *et al.*, 1993; Pottinger & Moran, 1993; Cnaani *et al.*, 2004; Verbeek *et al.*, 2008). For example, brook charr *Salvelinus fontinalis* (Mitchill 1814) are less sensitive to transport and net confinement stress (reduced ion loss) compared to lake trout *Salvelinus namaycush* (Walbaum 1792) (McDonald *et al.*, 1993). Furthermore, quantitative genetic studies have revealed a moderate to high degree of heritability of the cortisol response for different fishes including carp *Cyprinus carpio* L. 1758 [0·60, (Tanck *et al.*, 2001)] and *O. mykiss* [0·56 for North American lines (Weber *et al.*, 2008); 0·50 for European lines, (Fevolden *et al.*, 2002)]. Given such additive genetic components, stress resistance, and more specifically variation in stress-induced cortisol concentration, has been identified as a trait of interest for genetic improvement (Fevolden *et al.*, 1991; Lankford & Weber, 2006). Studies using selective breeding programmes for disease resistance or growth that aim to improve fish performance *via* a lower cortisol response, however, have met with limited success thus far (Lankford & Weber, 2006; Weber & Silverstein, 2007).

Another approach that can be considered for the genetic improvement of physiological traits is the production of hybrid crosses that may result in heterosis (*i.e.* hybrid vigour), which is the improved performance of first-generation progeny compared to parental lines (Falconer & Mackay, 1996). Heterosis is the most important non-additive effect on cross performance and is usually stronger when parental lines are genetically distant from each other (Shikano *et al.*, 2000; Wang & Xia, 2002). This phenomenon is now being used in improvement schemes concerning traits of interest in aquaculture, including growth rate, survival and salinity tolerance (Bentsen *et al.*, 1998; Shikano & Taniguchi, 2002; Bryden *et al.*, 2004; Hena *et al.*, 2005). Until now, very few studies have investigated the importance of heterosis on stress response in fishes (Campbell *et al.*, 1998; Bryden *et al.*, 2004).

The main objective of this study was to test for the occurrence and to quantify the importance of heterosis in the physiological stress response by comparing three pure strains of *S. fontinalis* and their F1 hybrids. More specifically, the effects of stress

induced by transportation, a common activity in aquaculture that often results in mortality, were investigated. A second objective was to estimate heritability values for primary (plasma cortisol) and secondary (plasma glucose, plasma osmolality and haematocrit) stress indicators for the first time in *S. fontinalis* and to compare the observed values with other fishes. In this way, the present study planned to evaluate the relative merits of hybrid crosses and selective breeding for improving the response of *S. fontinalis* to stress in an aquaculture context.

MATERIALS AND METHODS

SALVELINUS FONTINALIS STRAINS

Three genetically distinct strains of *S. fontinalis* (Martin *et al*., 1997) were used as parental lines. The Laval strain originates from a wild population of anadromous *S. fontinalis* from the Laval River (48° 44′ N; 69° 05′ W) on the north shore of the St Lawrence River estuary (QC, Canada). The fish used were third-generation breeders reared in captivity at the Station aquicole ISMER–UQAR (Rimouski, QC, Canada). The Rupert strain originates from a freshwater-resident wild population inhabiting the Rupert River system (51° 05′ N; 73° 41′ W) (QC, Canada). The fish used as breeders were also from the third generation produced in captivity at the Laboratoire régional en sciences aquatiques (LARSA, Université Laval, Québec, QC, Canada). Finally, the so-called domestic strain is the main one being used by the Québec fish farming industry, and it originates from two strains (Nashua and Baldwin). Breeders used in this study were obtained from the Pisciculture de la Jacques Cartier (Cap-Santé, QC, Canada). The two wild strains were selected for breed improvement because adults from these populations exhibit late sexual maturation and large adult size.

BREEDING DESIGN

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

FAMILY REARING

From egg incubation (January) to exogenous feeding (June), each family was incubated separately in individual trays, and each incubation tank contained 12 trays. Water temperature was maintained at 6° C during egg incubation and at 8° C after hatching. The photoperiod was set at 12L:12D (0700–1900 hours). In June, families were identified using different combinations of adipose and pelvic fin clippings and transferred to nine 3 m^3 tanks, with eight families pooled per tank. All families were brought to 2136 degree days by the end of the summer and maintained at 10° C in recirculating fresh water. Photoperiod followed

TABLE I. Mean ± s.e. body mass (M_B) and fork length (L_F) of the *Salvelinus fontinalis* breeders used to produce the different purebred and hybrid cross-types and M_B and L_F of the three purebred strains and their hybrids used as controls or for the stress challenge. Within each column, different lower case letters indicate significant differences among cross-types ($\alpha = 0.05$)

Cross	Dams			Sires			Controls			Stressed fish		
	n	M_B (kg)	L_F (cm)	n	M_B (kg)	L_F (cm)	n	M_B (g)	L_F (cm)	n	M_B (g)	L_F (cm)
D♀R♂	10	0.59 ± 0.02[ab]	35.7 ± 0.4[a]	10	0.63 ± 0.04[a]	37.7 ± 1.0[a]	20	41.9 ± 2.1[de]	15.7 ± 0.3[c]	20	49.3 ± 4.2[de]	16.5 ± 0.4[c]
D♀D♂	10	0.70 ± 0.02[c]	36.8 ± 0.4[a]	10	0.81 ± 0.03[a]	38.4 ± 0.7[a]	20	58.2 ± 5.5[e]	16.6 ± 0.5[c]	20	61.5 ± 5.3[e]	17.3 ± 0.5[c]
D♀L♂	10	0.78 ± 0.07[bcd]	38.1 ± 1.3[ab]	10	1.03 ± 0.12[ab]	44.0 ± 0.7[bc]	20	37.8 ± 3.5[cd]	15.0 ± 0.5[bc]	20	39.1 ± 4.0[cd]	15.4 ± 0.5[bc]
L♀D♂	10	0.97 ± 0.10[cd]	41.3 ± 0.7[b]	10	0.71 ± 0.03[a]	37.7 ± 0.4[a]	20	33.4 ± 2.4[cd]	14.7 ± 0.3[bc]	20	45.4 ± 4.1[cd]	16.4 ± 0.4[c]
L♀L♂	10	1.07 ± 0.08[d]	42.6 ± 0.9[b]	10	1.25 ± 0.06[bc]	44.8 ± 0.6[bc]	20	15.6 ± 1.0[a]	11.9 ± 0.3[a]	20	14.0 ± 0.7[a]	11.5 ± 0.2[a]
L♀R♂	10	1.16 ± 0.14[c]	42.2 ± 0.7[b]	10	0.85 ± 0.09[ab]	40.3 ± 1.3[ab]	20	24.5 ± 2.1[bc]	13.6 ± 0.4[ab]	20	31.9 ± 3.2[bc]	14.9 ± 0.5[bc]
R♀L♂	10	1.39 ± 0.21[bcd]	45.5 ± 2.0[b]	10	1.46 ± 0.17[c]	46.3 ± 0.6[a]	20	23.9 ± 2.3[b]	13.5 ± 0.4[ab]	20	21.3 ± 1.7[b]	13.1 ± 0.3[ab]
R♀R♂	10	0.47 ± 0.04[a]	35.7 ± 1.0[a]	10	0.77 ± 0.11[a]	40.3 ± 1.8[abc]	20	22.8 ± 1.5[b]	13.2 ± 0.3[ab]	20	22.4 ± 1.5[b]	13.2 ± 0.3[b]

D, domestic; L, Laval; R, Rupert.

n, the number of individuals.

the natural seasonal cycle (48° 3′ N), and fish were fed according to commercial charts with commercial pellets. In September, fish were transferred in transport bags (one family per bag) immediately to the Station aquicole ISMER−UQAR. Here, they were reared in ten 0·5 m³ indoor tanks, with six to eight families per tank, under natural temperature and photoperiod (48° 3′ N) conditions in running dechlorinated fresh water. Fish were fed daily (1% mass to body mass ration) with commercial dry pellets. No mortality difference was observed among cross-types during the whole rearing period. There was no disease occurrence, and prophylactic treatments (chloramines T) were applied following marking and M_B and L_F measurements.

STRESS EXPOSURE

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

SAMPLING PROCEDURES

All sampling was done between 1600 and 1900 hours to avoid bias due to endocrine circadian rhythms. Stressed and control fish were anaesthetized in MS-222 [0·16 g l^{-1} (3-aminobenzoic acid ethyl ester)], and M_B (to the nearest 0·1 g) and L_F (0·1 cm) were measured (Table I). Blood was collected by caudal puncture using ammonium-heparinized syringes. A small quantity of blood was transferred to capillary tubes for haematocrit determination, and the remainder was centrifuged at 7200 *g* for 3 min. The plasma was drawn off, quickly frozen in liquid nitrogen and then stored at −80° C until analysis. Plasma osmolality was measured with an advanced micro-osmometer 3MO (www.blockscientificstore.com), plasma glucose was measured by enzymatic determination (Alexander & Griffiths, 1993) and cortisol levels were measured using a MP Biomedicals cortisol ^{125}I RIA kit (www.mpbio.com).

STATISTICAL ANALYSES

Data normality and homogeneity of variance were tested with Kolmogorov−Smirnov and Brown−Forsythe tests, respectively. Plasma cortisol concentrations were log_{10} transformed to obtain normality. The variability between replicate transport bags was tested using ANOVA and was not significant. The different variables were analysed using two-way ANOVAs with cross-type, stress treatment and stress treatment × cross-type interaction as fixed effects. The effect of dam and sire origin (D, L or R) on each physiological variable after stress exposure was analysed using two-way ANOVAs with dam and sire origin as factors. The presence of heterosis or outbreeding depression was determined by the presence of a significant difference between the mean performance of hybrids compared to the mean performance of both parental strains (Bryden *et al.*, 2004). Heterosis was expressed when there was a lower stress response in hybrids compared to parental lines. *A posteriori* Tukey tests were used for mean comparisons when possible or replaced by Games and Howell tests when variances were not homogenous. The influence of M_B on variables was examined using M_B as a covariate in ANCOVA. Analyses were made using Statistica version 6.0 for Windows (www.statsoft.com). A significance level of $\alpha = 0.05$ was used in all statistical tests.

HERITABILITY ANALYSES

The breeding design was used to fit animal models (Lynch & Walsh, 1998) with the ASReml software (V2.0; www.vsni.co.uk; Gilmour *et al.*, 2006). Univariate analyses were used to disaggregate the phenotypic variance (V_P) of each trait for the whole fish population (including pure and hybrid crosses) into their additive genetic (V_A) and residual (V_R) variances. The model was: $y = \mu + C + A + e$, where y is the phenotypic observation, μ is the overall mean, C is the fixed effect of the cross-type, A is the random additive genetic effect and e is the random residual effect. The narrow-sense heritability (h^2) for each trait was estimated as the ratio of the additive genetic variance (V_A) to the total phenotypic variance (V_P): $h^2 = V_A V_P^{-1}$. The statistical significance of the additive genetic component for each trait was tested by re-running a restricted model where the additive variance was set to zero and then comparing the difference in the log-likelihood ratio between the original and the restricted model against the χ^2 distribution (d.f. = 1), where $\chi^2 = -2 \times$ difference in log likelihood.

RESULTS

PLASMA CORTISOL RESPONSE

A stress response was noted in every cross-type (stress treatment: $F_{1,300} = 1132$, $P < 0\cdot001$), as shown by a significant increase in cortisol between control and stressed fish (Fig. 1). The intensity of the cortisol response, however, was variable depending on the cross, with significant interactions observed between stress treatment and cross-types (stress treatment × cross-type: $F_{7,300} = 2\cdot2$, $P < 0\cdot05$; Fig. 1). All control fish had the same level of initial plasma cortisol (Fig. 1). The
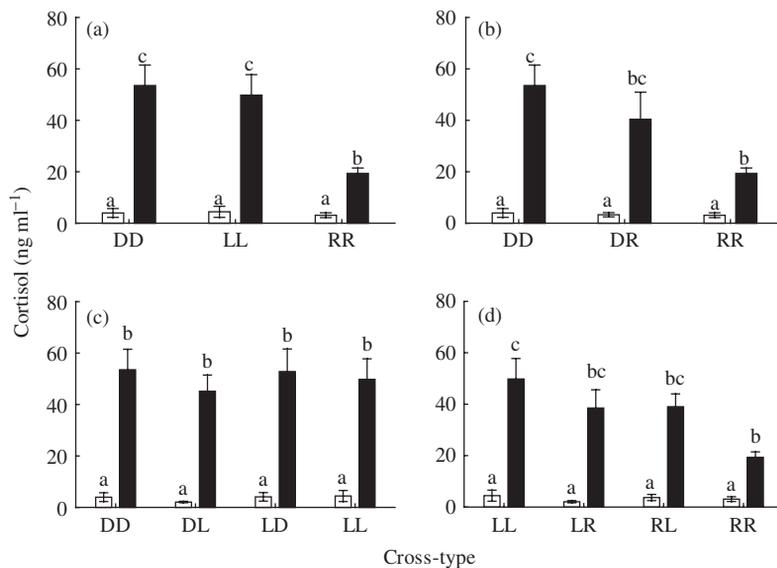

FIG. 1. Cortisol stress response (■) in the (a) three *Salvelinus fontinalis* purebred strains [domestic (D), Laval (L) and Rupert (R)] and hybrids between (b) D and R strains, (c) D and L strains and (d) L and R strain (□, controls). The first letter of the cross-type indicates the dam and the second letter the sire. Statistical analyses were made on log$_{10}$-transformed data, but results are presented as mean ± S.E. For each bar, $n = 20$. Different lower case letters indicate significantly different means ($\alpha = 0\cdot05$).

stress treatment in purebred crosses induced a significantly lower cortisol response in the R fish than in L and D fish, with the last two being similar [Fig. 1(a)]. In hybrids, when the R strain was used as either dam or sire, the post-stress cortisol level did not differ significantly from either parental line [Fig. 1(b), (d)]. In crosses involving the domestic and the L strains, all hybrids and parental lines showed similar cortisol responses [Fig. 1(c)]. These results are indicative of an additive response rather than a non-additive effect. The M_B had no significant effect on this trait [M_B (co-variable): $F_{1,300} = 1·7$, $P > 0·05$].

SECONDARY STRESS RESPONSE INDICATORS

A significant interaction was observed between stress treatment and cross-type for glucose concentration (stress treatment × cross-type: $F_{7,289} = 2·3$, $P < 0·05$). Plasma glucose concentrations were similar for all controls (Fig. 2), while they were significantly higher after stress exposure in all cross-types (Fig. 2). The glucose response was similar among the three purebred lines [Fig. 2(a)], and hybrids showed concentrations similar to their parental lines [Fig. 2(c), (d)]. The only exception was the $D_{\female}R_{\male}$ hybrid, which had a significantly higher glucose concentration after stress exposure [Fig. 2(b)], hence expressing outbreeding depression. Glucose concentration was 27% higher in this hybrid after stress exposure compared to the average
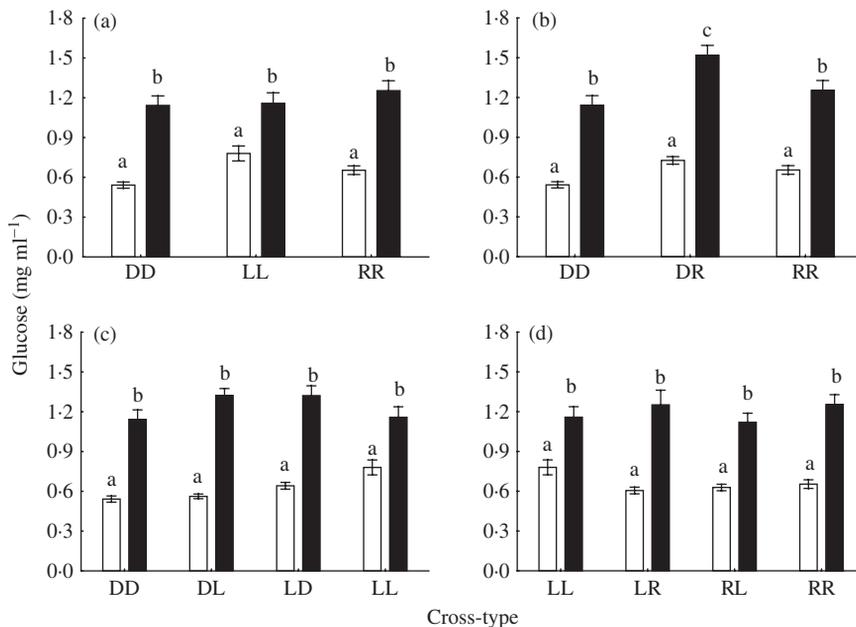

FIG. 2. Plasma glucose stress response (■) in the (a) three *Salvelinus fontinalis* purebred strains [domestic (D), Laval (L) and Rupert (R)] and hybrids between (b) D and R strains, (c) D and L strains and (d) L and R strains (□, controls). The first letter of the cross-type indicates the dam and the second letter the sire. Values are mean ± S.E. ($n = 20$). Different lower case letters indicate significantly different means ($\alpha = 0·05$).
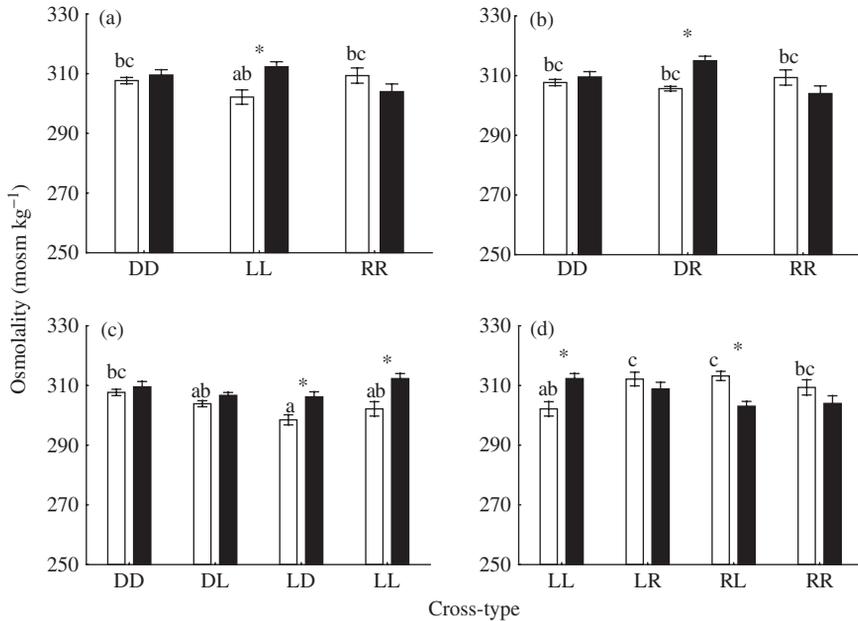
FIG. 3. Osmolality stress response (■) in the (a) three *Salvelinus fontinalis* purebred strains [domestic (D), Laval (L) and Rupert (R)] and hybrids between (b) D and R strains, (c) D and L strains and (d) L and R strains (□, controls). The first letter of the cross-type indicates the dam and the second letter the sire. Values are mean ± S.E. (*n* = 20). Different lower case letters indicate significantly different means among controls; ∗, significantly different means between control and stressed fish ($\alpha = 0.05$).

glucose concentration in parental lines. There was no significant co-factor effect for M_B [M_B (co-variable): $F_{1,289} = 2.2$, $P > 0.05$].

A significant interaction was observed between stress treatment and cross-type in the plasma osmolality response to transport stress (stress treatment × cross-type: $F_{7,274} = 8.0$, $P < 0.001$; Fig. 3). In purebred lines, controls were not different [Fig. 3(a)]. Following stress exposure, L fish had significantly higher plasma osmolality levels than controls, while osmolality did not vary in the other two purebred lines [Fig. 3(a)]. Pre-stress levels of plasma osmolality were similar to both parental lines in the $D_{\female}R_{\male}$ and $D_{\female}L_{\male}$ hybrids [Fig. 3(b), (c)], similar to the L line in the $L_{\female}D_{\male}$ hybrid [Fig. 3(c)] and similar to the R line in hybrids between the R and the L lines [Fig. 3(d)]. After stress exposure, there was a significant increase in plasma osmolality in the $D_{\female}R_{\male}$ hybrid, while no change was observed in the parental lines [Fig. 3(b)]. The reverse was observed in the $R_{\female}L_{\male}$ hybrid, with a significant decrease in plasma osmolality [Fig. 3(d)]. As with the R line, no osmolality change was observed in the $L_{\female}R_{\male}$ hybrids [Fig. 3(d)], and hybrids between the D and the L strains behaved in a way similar to their maternal strain [Fig. 3(c)]. The interaction between stress treatment and cross-type was significant for the blood haematocrit response (stress treatment × cross-type: $F_{7,278} = 2.3$, $P < 0.05$). Blood haematocrit was similar among controls (mean ± S.E. 36 ± 1%) and increased only in the domestic line after stress exposure ($D_{\female}D_{\male}$: 34 ± 1% in controls *v.* 42 ± 1% after stress exposure). For both plasma osmolality and blood haematocrit, the M_B co-factor

TABLE II. Genetic components of the different traits in the stress responses in *Salvelinus fontinalis*. Estimates of total phenotypic (V_P), additive (V_A) and residual (V_R) variance components and heritability (h^2) are given with their $\pm$ s.e. *P*-values were obtained from a likelihood ratio test

	n	V_P	V_R	V_A	h^2	*P*
Cortisol	159	0.14 ± 0.03	0.06 ± 0.02	0.08 ± 0.04	0.60 ± 0.20	<0.05
Glucose	158	0.17 ± 0.04	0.07 ± 0.02	0.11 ± 0.06	0.61 ± 0.20	<0.05
Osmolality	148	58.92 ± 7.04	58.92 ± 7.04	0.00	0.00	>0.05
Haematocrit	146	0.004 ± 0.001	0.002 ± 0.001	0.002 ± 0.002	0.46 ± 0.25	>0.05

n, the number of individuals.

was significant [plasma osmolality, M_B (co-variable): $F_{1,274} = 8.7$, $P < 0.01$; blood haematocrit, M_B (co-variable): $F_{1,278} = 7.9$, $P < 0.01$] but correlations were weak ($r = 0.15$ for both).

HERITABILITY

Significant additive genetic variance and heritability were obtained at both stress response levels for the whole population. Heritability estimates for cortisol ($h^2 = 0.60 \pm 0.20$) and glucose ($h^2 = 0.61 \pm 0.20$) following stress exposure were high and significant (Table II), while estimates were not significant for osmolality ($h^2 = 0.00 \pm 0.00$) or haematocrit ($h^2 = 0.46 \pm 0.25$) (Table II).

PARENTAL ORIGIN EFFECTS

Dam and sire origin significantly affected the stress response depending on the trait as was the case for heritability, the parental origin effect was strong for cortisol (Table III). The results for the secondary response, however showed different tendencies: (1) there were significant effects of both dam and sire origin in the cortisol response, with fish issued from the R strain having lower plasma cortisol than other fish, (2) no significant dam or sire effect was observed for the glucose response and (3) there was a significant dam origin effect on the osmolality and haematocrit stress responses (Table III). Progeny of R dams had lower plasma osmolality following stress exposure than progeny of the other two strains when used as dams, and progeny of L dams had lower haematocrit after stress exposure than when domestic dams were used.

DISCUSSION

The main objectives were to determine whether heterosis occurred and to estimate the heritability of primary and secondary stress indicators in *S. fontinalis*. While the results revealed no clear evidence of heterosis, relatively high heritability was found for endocrine and physiological responses. A third objective was to compare the stress response between strains of *S. fontinalis*. Interstrain differences have been previously reported between unselected lines of *B. splendens* and also between lines selected

TABLE III. Dam and sire origin effects on the different traits after stress exposure in *Salvelinus fontinalis*. Physiological traits are expressed as mean ± s.e. Different lower case letters indicate significant differences among cross-types ($\alpha = 0.05$)

	Dam				Sire			
	Domestic	Laval	Rupert	P	Domestic	Laval	Rupert	P
Cortisol (ng ml^{-1})	46·39 ± 4·85[b]	47·06 ± 4·60[b]	28·96 ± 3·07[a]	<0·05	53·19 ± 5·85[b]	44·78 ± 3·78[b]	32·78 ± 4·40[a]	<0·01
Glucose (mg ml^{-1})	1·33 ± 0·04	1·24 ± 0·05	1·19 ± 0·05	>0·05	1·23 ± 0·05	1·21 ± 0·04	1·34 ± 0·05	>0·05
Osmolality (mosm kg^{-1})	310·4 ± 0·9[b]	309·1 ± 1·1[b]	303·5 ± 1·5[a]	<0·01	307·9 ± 1·3	307·6 ± 1·0	309·9 ± 1·3	>0·05
Haematocrit (%)	0·40 ± 0·01[b]	0·37 ± 0·01[a]	0·37 ± 0·01[ab]	<0·01	0·39 ± 0·01	0·38 ± 0·01	0·37 ± 0·01	>0·05

for different response to stress in *O. mykiss* and *S. salar* (Fevolden *et al*., 1991; Pottinger, 2006; Verbeek *et al*., 2008). In these studies, the stress cortisol response varied by 1·25 to two times when the most sensitive population is compared to the least sensitive one. The results from the present study indicate a similar range, with the Rupert strain response being about half those of the other purebred strains.

PUREBRED LINES

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

NON-ADDITIVE GENETIC EFFECTS

No non-additive components seemed to influence the cortisol response; this is similar to findings on other species [channel catfish *Ictalurus punctatus* (Rafinesque 1818), Bosworth *et al*., 2004; Chinook salmon *Oncorhyncus tshawytsha* (Walbaum 1792), Bryden *et al*., 2004]. Studies on hybrids have rarely provided evidence of non-additive effects, but they generally focussed on survival or cortisol response (Bulger & Schultz, 1982; Bosworth *et al*., 2004; Bryden *et al*., 2004). Heterosis related to survival time (tertiary response), however, was reported in F1 hybrids after salinity stress in *Poecilia reticulata* Peters 1859 (Chiyokubo *et al*., 1998) and heat stress in the hybrid *Poeciliopsis monacha* Miller 1960 × *Poeciliopsis occidentalis* (Bain & Girard 1853) (Bulger & Schultz, 1982).

A weak but significant non-additive component was present at the physiological level (secondary response), especially for plasma glucose concentration in the $D_♀R_♂$ hybrid. A non-additive component was also observed for plasma osmolality in $D_♀R_♂$ and $R_♀L_♂$ hybrids, but this is more difficult to interpret for the $D_♀R_♂$ hybrid, as previously mentioned. The observations of non-additive components only at the secondary response level reveal the presence of genetic divergence in purebred strains at the physiological level rather than a neuroendocrine response to stress stimuli. The extents of non-additive genetic phenomena are thought to be principally linked to the genetic distance between parental lines (Falconer & Mackay, 1996; Tymchuk *et al*., 2007). If the lines are too genetically distant or adapted to their own environment, hybrids can show outbreeding depression with a breakdown of genetic complex associations; on the other hand, when the genetic distance between parental strains is closer, hybrids can express heterosis (Falconer & Mackay, 1996; Shikano *et al*., 2000; Cooke *et al*., 2001). The results do not support any of these expectations according to genetic distance: (1) the Laval and Rupert strains were the most

genetically distant strains (Martin *et al*., 1997), and one of their hybrids ($R_{\female}L_{\male}$) expressed a response significantly different from the parental responses for osmolality and (2) the $D_{\female}R_{\male}$ hybrid expressed outbreeding depression (glucose), while the two parental lines were more genetically similar. In addition, the results obtained for the other hybrids do not support the hypothesis that the genetic distance would be the main effect involved in non-additive expression in crosses. Other effects related to genetic architecture (*e.g.* epistasis, pleitropy or genetic linkage) should be explored to explain the results. Overall, the presence of non-additive genetic effects only in secondary stress responses suggests that the use of hybrids to improve transport stress resistance in aquaculture has limited potential.

ADDITIVE GENETIC EFFECTS

The primary response to stress, *i.e.* cortisol response, seems to be principally under additive genetic control. The plasma cortisol concentration in hybrids was always similar to both parental lines. Both dam and sire origin significantly affected this trait, indicating the importance of an additive genetic basis underlying this stress response. Other studies on hybrids also revealed additive effects on plasma cortisol level after exposure to stress: Bryden *et al*. (2004) exposed wild and farm *O. tshawytscha* hybrids and purebred crosses to an aerial emersion stressor, and the cortisol response in hybrids was equal to both parental lines. The high additive component for cortisol regulation translated into high and significant heritability estimate for this trait ($h^2 = 0.60 \pm 0.20$). The cortisol response to stress is already used for genetic improvement in other fish species, especially in *O. mykiss*, in which heritability values similar to those obtained in the present study have been documented in the F1 generation (h^2 ranging from 0.41 to 0.56 depending on strain origin) (Pottinger & Carrick, 1999; Fevolden *et al*., 2002; Overli *et al*., 2005; Weber & Silverstein, 2007; Weber *et al*., 2008). The selection procedure for stress response in *O. mykiss* was based on the mean post-stress plasma cortisol response across five episodes of confinement stress testing on parental lines, with the highest responding (HR) or lowest responding (LR) individuals used to produce the next generation. This breeding programme was repeated several times to obtain F2 and F3 generations with improved stress resistance and other possibly related traits, such as increased growth or disease resistance (Pottinger & Carrick, 1999; Overli *et al*., 2005; Ruiz-Gomez *et al*., 2008). The results from the present study suggest that such a programme could also be applied in *S. fontinalis*.

For the secondary stress response, plasma glucose concentration also displayed significant heritability estimates. This trait had higher heritability ($h^2 = 0.61 \pm 0.20$) than values reported in previous studies on androgenetic *C. carpio* (0.19; Tanck *et al*., 2001) *S. salar* (0.03; Fevolden *et al*., 1993) and *O. mykiss* (0.07; Fevolden *et al*., 1993). The low heritability observed in *C. carpio* could be related to the androgenetic design, *i.e.* the UV irradiation and heat shock treatment might induce additional environmental variation due to embryonic damage caused by the androgenetic shock treatment and therefore reduce heritability (Tanck *et al*., 2001). On the other hand, the estimates obtained in the present study could have been inflated due to the full-sib design, which may include other sources of variance including maternal effects (Falconer & Mackay, 1996; Pante *et al*., 2002). Previous studies in *S. fontinalis,* however, revealed that while maternal effects are apparent during the very first stages

of development, they vanish within several months following hatching (Perry *et al*., 2004, 2005). This suggests that maternal effect should have a limited influence on the results obtained. No significant heritability was found for osmolality or haematocrit response. Until now, no study has documented the heritability of osmolality variations related to stress resistance, but a very low heritability for haematocrit (0·072) was reported in clonal lines of ayu *Plecoglossus altivelis* (Temminck & Schlegel 1846) (DelValle *et al*., 1996).

In summary, the significant heritability of stress response at both the primary (cortisol) and secondary (glucose) levels indicates a good potential for selective breeding and genetic improved resistance to transport stress in *S. fontinalis* and particularly so for the Rupert strain. Future work should aim at determining whether the difference expressed among strains is the result of global stress sensitivity variations or if some strains are more sensitive than others to different types of stress. On the other hand, hybridization does not seem to be a promising avenue for improving stress resistance in *S. fontinalis*. Nevertheless, it would be worth further investigating this issue by comparing strains specifically selected for different sensitivity to stress response which was not the case here. Thus, fixation of alleles related to the stress response in different strains could produce different, non-additive physiological effects in mixed progenies.

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