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Divergence in physiological factors affecting swimming performance between anadromous and resident populations of brook charr Salvelinus fontinalis

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Abstract:	In this study, an anadromous strain and a freshwater-resident strain of brook charr Salvelinus fontinalis as well as their reciprocal hybrids were reared in a common environment and submitted to swimming tests combined with salinity challenges. The critical swimming speeds (Ucrit) of the different crosses were measured in both fresh (FW) and salt water (SW), and the variations in several physiological traits (osmotic, energetic, and metabolic capacities) that are predicted to influence swimming performance were documented. Anadromous and resident fish reached the same Ucrit in both FW and SW, with Ucrit being 14% lower in SW compared to FW. However, the strains seemed to use different underlying strategies: the anadromous strain relied on its streamlined body shape and higher osmoregulatory capacity, while the resident strain had greater citrate synthase (FW) and lactate dehydrogenase (FW, SW) capacity and either greater initial stores or more efficient use of liver (FW, SW) and muscle (FW) glycogen during exercise. Compared to R♀L♂ hybrids, L♀R♂ hybrids had a 20% lower swimming speed, which was associated with a 24% smaller cardio-somatic index and higher physiological costs. Thus swimming performance depends on cross direction (i.e., which parental line was used as dam or sire). The study thus suggests that divergent physiological factors between anadromous and resident S. fontinalis may result in similar swimming capacities that are adapted to their respective lifestyles.

Divergence in physiological factors affecting swimming performance between anadromous and resident populations of brook charr *Salvelinus fontinalis*

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ABSTRACT

In this study, an anadromous strain and a freshwater-resident strain of brook charr *Salvelinus fontinalis* as well as their reciprocal hybrids, were reared in a common environment and submitted to swimming tests combined with salinity challenges. The critical swimming speeds (U_{crit}) of the different crosses were measured in both fresh (FW) and salt water (SW), and the variations in several physiological traits (osmotic, energetic, and metabolic capacities) that are predicted to influence swimming performance were documented. Anadromous and resident fish reached the same U_{crit} in both FW and SW, with U_{crit} being 14% lower in SW compared to FW. However, the strains seemed to use different underlying strategies: the anadromous strain relied on its streamlined body shape and higher osmoregulatory capacity, while the resident strain had greater citrate synthase (FW) and lactate dehydrogenase (FW, SW) capacity and either greater initial stores or more efficient use of liver (FW, SW) and muscle (FW) glycogen during exercise. Compared to R♀L♂ hybrids, L♀R♂ hybrids had a 20% lower swimming speed, which was associated with a 24% smaller cardio-somatic index and higher physiological costs. Thus swimming performance depends on cross direction (*i.e.*, which parental line was used as dam or sire). The study thus suggests that divergent physiological factors between anadromous and resident *S. fontinalis* may result in similar swimming capacities that are adapted to their respective lifestyles.

Key words: swimming performance; metabolism; local adaptation; hybrids

INTRODUCTION

During their life cycle, many fishes species undergo migrations between habitats that are essential for completing their life cycle (*e.g.,* reproductive, nursery, and feeding habitats). These movements occur on temporal and spatial scales ranging from daily to annual and from a few metres to thousands of kilometres (McDowall, 1997; Klemetsen *et al.*, 2003; Fraser & Bernatchez, 2005; Kitano *et al.*, 2012). The environmental conditions encountered largely determine the physiological cost associated with these migratory movements.

In salmonids, swimming ability and support capacities (*e.g.*, oxygen transport, cardiovascular performance, and energy metabolism) fundamentally contribute to the success of migratory movements (Eliason *et al.*, 2011; Eliason & Farrell, 2016). In these species, migratory behaviour involves rapid transitions between freshwater and seawater, and osmoregulatory ability is a strong determinant in the success of such movements (McDowall, 1997; Peake *et al.*, 1997; Claireaux & Audet, 2000; Boula *et al.*, 2002; Wagner *et al.*, 2006). Links between swimming ability and capacity to maintain body fluid osmolality have been amply documented in fishes (Brauner *et al.*, 1992; Brauner *et al.*, 1994; Nelson *et al.*, 1996; McKenzie *et al.*, 2001a; McKenzie *et al.*, 2001b). For instance, in Coho salmon *Oncorhynchus kisutch* (Walbaum 1792) smolts and juvenile Adriatic sturgeon *Acipenser naccarii* (Bonaparte 1836), an acute increase in water salinity associated with an increase of plasma ions and osmolality was found to be directly related to a reduction in maximum sustainable swimming speed (Brauner *et al.*, 1992; Brauner *et al.*, 1994; McKenzie *et al.*, 2001a; McKenzie *et al.*, 2001b). Conversely, the lack of significant effects of ambient salinity on European seabass *Dicentrarchus labrax* (L. 1758) swimming and cardiac performance was linked to an exceptional capacity of this

64 species to maintain plasma osmolality and tissue water content when exposed to an acute change in
65 ambient salinity (Chatelier *et al.*, 2005).
66
67 In salmonids, migratory behaviour has evolved as a mandatory phase in the life cycle of some
68 species whereas it is optional in others (McDowall, 1997; Klemetsen *et al.*, 2003; Fraser & Bernatchez,
69 2005; Thériault *et al.*, 2007; Arai & Goto, 2008). In brook charr *Salvelinus fontinalis* Mitchill 1814, the
70 ancestral form of anadromy is now facultative (Castric & Bernatchez, 2003; Curry *et al.*, 2010), and
71 different migratory patterns exist depending on the biotic and abiotic conditions in the native
72 environment of a population (Castric & Bernatchez, 2003). The anadromous *S. fontinalis* population of
73 the Laval River (L) (48° 44' N; 69° 05' W) on the north shore of the St. Lawrence estuary migrates to
74 freshwater for reproduction and overwintering and to salt water in summer for feeding. These fish can
75 thrive in habitats encompassing a wide range of environmental conditions—from low to high salinity (1
76 to 34), temperature (5 to 18°C), and water velocities (Boula *et al.*, 2002; Curry *et al.*, 2006). The
77 Rupert population (R) is a strictly freshwater resident *S. fontinalis* population originating from the
78 Rupert River (51° 05' N; 73° 41' W) near Lake Nemiscau (near James Bay in NW Québec). These fish
79 always live in cold freshwater and migrate from the river to lakes for reproduction (MAPA-Pêcheries,
80 1992). In addition to living in two different environments and having different lifestyles, previous
81 genetic studies revealed a pronounced genetic differentiation between these two populations
82 ($F_{st} = 0.427 \pm 0.020$; Martin *et al.*, 1997), as well as important differences in gene expression when
83 reared in a same environment (Bougas *et al.*, 2010). However, it is not known whether these
84 differences are accompanied by a divergence in their swimming capacity.
85
86 Previous studies on salmonids have revealed that different lifestyles among species or populations
87 may result in differences in their swimming ability (Taylor & McPhail, 1985; Hawkins & Quinn, 1996;
88 Peake *et al.*, 1997). In Atlantic salmon *Salmo salar* (L. 1758), anadromous individuals possess greater

89 sustained swimming ability than landlocked ones, possibly related to their different morphology (the
90 anadromous form has a more fusiform body shape than the landlocked one) and migratory histories
91 (Peake *et al.*, 1997). When swimming tests were conducted in common environments, the differences
92 between populations remained (Taylor & Foote, 1991), suggesting a genetic basis for swimming
93 performance and thus a potential for evolutionary adaptation. In threespine stickleback *Gasterosteus*
94 *aculeatus* (L. 1758), comparisons of swimming performance in freshwater resident and anadromous
95 populations, both in Europe and North America, have shown that anadromous fish had a greater
96 swimming performance than the freshwater residents (Tudorache *et al*., 2007, European populations;
97 Dalziel *et al*., 2011, North American populations). In the North American populations, this difference
98 is genetically based (Dalziel *et al*., 2011). Understanding the genetic and physiological bases of
99 evolutionary change in swimming capacity in *S. fontinalis* could provide further insight into the
100 functional bases of differential adaptation in swimming capacity of fishes (Odell *et al.*, 2003; Collin &
101 Fumagalli, 2011; Dalziel *et al.*, 2011).
102
103 Hybridization between different populations may also provide important information on the genetic
104 basis of swimming performance and the degree of divergence between populations. For example,
105 measuring traits in F1 hybrids could reveal the relative importance of additive or non-additive genetic
106 effects in the expression of performance (Dalziel *et al.*, 2011). When populations are genetically closer,
107 hybrids tend to express additive genetic effects and show intermediate performance compared to their
108 parental lines. On the contrary, when populations are genetically divergent and adapted to their own
109 environments, hybrids may express non-additive genetic effects due to complex genetic associations
110 (Falconer & Mackay, 1996; Edmands, 1999; Cooke *et al.*, 2001; Cooke & Philipp, 2005; Stelkens *et*
111 *al.*, 2009). Non-additive genetic effects have been reported for various morphological and
112 physiological traits such as size, survival, and other fitness-related traits in rainbow trout *Oncorhynchus*
113 *mykiss* (Walbaum 1792) (Tymchuk *et al.*, 2009), *O. kisutch* (Emlen, 1991), and *S. fontinalis* (Granier *et*

114 *al.*, 2011; Crespel *et al.*, 2012), and also in swimming performance in largemouth bass *Micropterus*
115 *salmoides* (Lacepède 1802) (Cooke *et al.*, 2001). The occurrence of non-additive genetic effects
116 controlling fitness-related traits thus provide further evidence for evolutionary divergence among the
117 populations studied. However, the occurrence of non-additive genetic effects in swimming
118 performance and its underlying physiological basis among populations with different migratory
119 lifestyles has rarely been investigated.
120
121 Whether anadromous fish are better swimmers than freshwater residents has been tested,
122 hypothesizing that this trait would be a major fitness component in migratory fish. In addition to
123 condition factor and energy reserve levels, a whole range of physiological factors can affect fish
124 swimming capacity, thus the measurement of these variables gives information on their relative
125 contributions. Blood oxygen-carrying capacity was inferred from blood hematocrit and haemoglobin
126 concentration, leading to the calculation of the mean cellular haemoglobin concentration. The
127 capacities of experimental populations to mobilize energy reserves to fuel working muscles were
128 compared by measuring blood glucose as well as liver and white muscle glycogen content. For the
129 same reason, white muscle and heart pyruvate and lactate concentrations were also assessed. The
130 activities of white muscle lactate dehydrogenase (LDH) and citrate synthase (CS) were measured
131 because these enzymes are important regulators of aerobic and anaerobic metabolism responding to
132 substrate/product ratios. These measurements provided insight into the relative contribution of aerobic
133 vs. anaerobic pathways to meet the energy needs associated with swimming. Since the capacity to
134 maintain plasma osmotic and ionic characteristics is a key factor affecting fish swimming capacity,
135 gill Na+K+ATPase activity was also assessed.
136
137 The occurrence of non-additive effects in the hybrids was investigated to obtain additional insight
138 into the genetic divergence between anadromous and resident strains. For this, two alternative

hypotheses were tested: 1) non-additive effects are present in hybrids, indicating a divergence for swimming performance between the two populations of origin and creating complex genetic associations during adaptation; or 2) the hybrids do not express non-additive effects, indicating that swimming performance is supported by compatible genes in the two populations of origin.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Experiments were conducted using two strains of wild *S. fontinalis* (Laval and Rupert) and the corresponding hybrid crosses. Breeders were third generation fish produced in captivity at the Station aquicole (ISMER-UQAR, Rimouski, QC, Canada) and at the Laboratoire de Recherche en Sciences Aquatiques (LARSA, Université Laval, Québec, QC, Canada). Four cross-types were produced during winter 2005: ♀ Laval × ♂ Laval ($L_{♀}L_{♂}$), ♀ Rupert × ♂ Rupert ($R_{♀}R_{♂}$), ♀ Laval × ♂ Rupert ($L_{♀}R_{♂}$), and ♀ Rupert × ♂ Laval ($R_{♀}L_{♂}$) (Fig. 1). For each cross-type, 10 full-sib families were obtained through single-pair mating. All families were reared under similar conditions in recirculating freshwater (FW) at LARSA from egg incubation (January) to exogenous feeding (at the end of the summer). Water temperature was maintained at 6°C during egg incubation and at 8°C after hatching. Photoperiod followed the natural seasonal cycle, and fish were fed according to commercial charts.

In September, fish were transferred to the Station aquicole ISMER-UQAR, where they were reared under natural temperature and photoperiod conditions in running dechlorinated FW. They were fed daily (ration of 1% food weight [g]/total fish wet mass [g]) with commercial dry pellets. In March, 10

163 fish from each family (100 fish per cross-type) were tagged using fingerling tags (Floy Tag Inc.,
164 www.floytag.com) (Fig. 1).
165
166 THE FLUME
167
168 The swimming tests were performed using a circular flume (with a linear swimming section)
169 designed to provide non-turbulent water flow (Redjah *et al.*, 2010). Briefly, a variable-speed motor
170 propelled the water at a constant velocity. Plastic honeycomb structures and deflectors were inserted in
171 the circulation loop upstream from the swimming chamber (23 × 37 × 22.3 cm) to promote rectilinear
172 flow and a uniform velocity profile. An acoustic Doppler velocimeter (type 16 MHz MicroADV,
173 Sonteck, www.sontek.com) was used to calibrate water velocity to voltage output from the motor
174 controller. The flume was supplied with fully aerated and thermoregulated (6.8 ± 0.3°C) water at a flow
175 rate of 10 l min^{-1}.
176
177 VALIDATION TEST AND CRITICAL SWIMMING SPEED PROTOCOL
178
179 To validate the swimming challenge procedure, two subgroups of 15 tagged fish per cross-type were
180 submitted to three consecutive swim tests in FW with a 4 h recovery period between tests 1 and 2 and a
181 16 h recovery period between tests 2 and 3, which is in line with the capacity of salmonids to fully
182 recover from exhaustion (45 to 90 min; Jain *et al.*, 1998; Lee *et al.*, 2003; Tierney & Farrell, 2004).
183 Cross-types were tested separately, with subgroups of fish swimming together in each trial (Table I).
184 The repeatability of individual performances was confirmed (Table II, $P > 0.05$) as was the fish
185 swimming performance ranking (data not shown; $P > 0.05$).
186
187 Following transfer into the swimming chamber, fish were left undisturbed for 30 min at a water

188 speed of 5.5 cm s^{-1} (*i.e.*, 0.5 standard length s^{-1} [L_S s^{-1}]). Following this acclimation period, fish were
189 submitted to a stepwise increase of water velocity from 5.5 to 11.0 to 16.5 cm s^{-1} at 5 min intervals, and
190 then to 22.0, 27.5, 33.0, 38.5, 44.0, 49.5, and, in some cases, 55.0 cm s^{-1} at 15 min intervals. Fish were
191 considered to be fatigued when they were unable to remove themselves from the screen situated
192 downstream from the swimming chamber. At that time, fish were removed from the swim chamber,
193 identified (tag reading), and placed in their original rearing tank. The corresponding water velocity and
194 time were recorded. The critical swimming speed (U_{crit}, L_S s^{-1}) was calculated according to Brett (1964)

195 $$U_{crit} = [U + (T / T_i \times U_i)] / L_S, \quad (1)$$

196 where U is the highest velocity maintained for the whole interval (cm s^{-1}), T is the time elapsed at
197 fatigue velocity (s), T_i is the prescribed interval time between each speed increment (300 s or 900 s), U_i
198 is the velocity increment (5.5 cm s^{-1}), and L_S is the fish standard length (cm). No correction for
199 blocking effect was applied since the total cross-sectional area of the fish did not exceed 5% of the
200 swimming chamber (Bell & Terhune, 1970).
201
202 EVALUATION OF SWIMMING CAPACITY
203
204 Following the assessment of measurement repeatability, the fish used for the validation tests were
205 directly transferred into salt water (SW; salinity 20, 6.8 ± 0.3°C). Salinity was adjusted by mixing St.
206 Lawrence estuarine water (salinity 31–32) with dechlorinated FW before it entered rearing tanks. After
207 a 48 h acclimation period, fish subgroups were submitted to the U_{crit} test as described above (Table I).
208 As one fish reached exhaustion, it was rapidly removed from the flume and anaesthetized in 3-
209 aminobenzoic acid ethyl ester (MS-222; 0.12 g l^{-1}) until opercular movements ceased (~ 1.5 to 2 min)
210 for blood and tissue samplings. Control fish were submitted to the same U_{crit} procedure described above
211 in FW, but only one group of 10 fish per cross-type swam together for these trials (Table I). Fish were
212 not fed for 48 h before their transfer to the swimming chamber. To avoid circadian bias in hormonal

measurements, SW and FW U_{crit} tests began at 1400 hours and were completed by 1630 hours.

BLOOD AND TISSUE SAMPLING

Following measurement of standard length (L_S to the nearest 0.1 cm) and body mass (M_B to the nearest 0.1 g) (Table III), blood was drawn by caudal puncture using ammonium-heparinized syringes. A small quantity of blood was kept for haematocrit and haemoglobin measurements, and the remainder was centrifuged at 7200 *g* for 3 min. Plasma aliquots were frozen in liquid nitrogen and stored at -80°C for further analyses. Gill filaments, liver, heart, and three pieces of epaxial muscle (one for each biochemical analysis) were excised, and liver and heart wet weight were recorded. Tissue samples were immediately frozen on dry ice and then stored at -80°C prior to analysis. An additional piece of epaxial dorsal muscle was excised, weighed, and dried for 72 h at 70°C for calculation of water content. Because body shape can affect swimming performance, condition factor (*CF*) was estimated according to the equation $M_B / L_S^3 \times 100$.

Plasma osmolality was measured with an Advanced Micro-osmometer (model 3MO, Advanced Instruments Inc., www.aicompanies.com), blood haemoglobin concentration was determined by Drabkin's method (Drabkin & Austin, 1935), plasma glucose was measured by enzymatic determination (Alexander & Griffiths, 1993), and cortisol levels were measured using a cortisol ^{125}I RIA kit (MP Biomedicals, www.mpbio.com). Mean cellular haemoglobin concentration (MCHC) was calculated using haematocrit data. Gill Na^+K^+ATPase capacity was measured using the micro-method described in Seigler *et al.* (1996).

Muscle and liver glycogen contents were determined according to the amyloglucosidase digestion method (Carr & Neff, 1984) followed by glucose concentration determination. Heart lactate, heart

pyruvate, white muscle lactate, and white muscle pyruvate concentrations were measured using enzymatic assays (Henry, 1968). Muscle samples were weighed and homogenized in 10 volumes of cold 100 mM imidazole-HCl buffer (pH 7.4), and LDH and CS capacity were measured according to Le François and Blier (2003). The Michaelis constant (K_m) was evaluated using different substrate concentrations, *i.e.*, from 0.01 to 0.5 mM oxaloacetate for CS and from 0.25 to 1 mM pyruvate for LDH, and calculated using a non-linear regression procedure (GraphPad Prism v.5, GraphPad Software Inc., www.graphpad.com).

STATISTICAL ANALYSES

It was assumed that fish were observed independently and that the number of degrees of freedom in the statistical analysis should be the number of fish. This was supported by the repeatability of individual performances (consecutive swim trials on the same groups of fish; Table II, $P > 0.05$) as well as fish swimming performance ranking (data not shown; $P > 0.05$).

Spearman rank order correlation and analysis of variance (ANOVA) with repeated measures were used to determine the repeatability of fish swimming performance rank. Normality and homogeneity of variances were verified by Kolmogorov-Smirnov and Brown-Forsythe tests, respectively. Muscle pyruvate concentration data were not normally distributed, so data were ranked and statistical procedures were applied on ranks (Quinn & Keough, 2002). Cortisol data were log transformed and lactate/pyruvate ratio data were square-root transformed to avoid heteroscedasticity. The different variables were analyzed using two-way analyses of covariance (ANCOVA) with salinity and cross-type as fixed effects and body mass as the covariable. If no covariance effect was found, a two-way ANOVA was run. The presence of non-additive effects was determined by the presence of significant differences between the mean trait values of hybrids compared to the mean traits of both parental

strains (Bryden *et al.*, 2004). When significant factor effects were found, *a posteriori* Tukey comparison of means tests (α=0.05) were used (Sokal & Rohlf, 1981). For those variables for which transformations failed to give homogeneity of variances, the Games and Howell test was used (Sokal & Rohlf, 1981). The least significant difference (LSD) test was used for muscle pyruvate concentration. All statistical analyses were performed with Statistica software (Statsoft v.6, www.statsoft.com).

RESULTS

The different cross-types used in this study were significantly different in terms of length and body mass even though they were raised under similar conditions and were the same age (Table III). *CF* was 20% lower in anadromous *S. fontinalis* ($L_{♀}L_{♂}$) than in resident fish ($R_{♀}R_{♂}$) (Table III). *CF* of $R_{♀}L_{♂}$ hybrids was similar to the paternal line ($L_{♀}L_{♂}$), while that of $L_{♀}R_{♂}$ hybrids was intermediate compared to parental lines. The cardio-somatic indexes (I_C) of the two purebred strains were similar and intermediate to those of the hybrids, with $R_{♀}L_{♂}$ having a higher I_C than $L_{♀}R_{♂}$ hybrids (Table III).

SWIMMING CHALLENGES

Critical swimming speed varied according to both cross-type and salinity with no significant interaction between the two factors, and body mass did not influence the critical swimming speed (Table IV). While U_{crit} values were similar in pure crosses of the anadromous and resident strains, swimming performance was 18% lower in $L_{♀}R_{♂}$ compared to the reciprocal $R_{♀}L_{♂}$. Also, swimming performance was significantly higher in FW (3.50 ± 0.13 L_S s^{-1}) compared to SW (3.00 ± 0.07 L_S s^{-1}) (Fig. 2).

STRESS AND OSMOTIC RESPONSE

288

289 Cortisol concentration was similar among all groups that underwent the swim challenge both in FW
290 and SW (Table IV), with an overall mean of 6.25 ± 0.60 μg dl^{-1}. Even though significant treatment
291 effects were found (Table IV), multiple comparison tests did not indicate differences in plasma glucose
292 between the different cross-types and salinity groups. The overall mean plasma glucose was 0.90 ±
293 0.04 mg ml^{-1}.

294

295 Muscle water content varied according to cross-type and salinity with no significant interaction
296 between the two (Table IV), and it was negatively correlated to body mass. The L♀L♂ fish had
297 significantly higher muscle water content (~1.7%) compared to fish from the other cross-types (Table
298 IV). Overall, muscle water content was close to 2% lower in fish challenged in SW than in fish
299 challenged in FW. A significant interaction between cross-type and salinity was observed for plasma
300 osmolality as was a significant negative body mass covariance effect (Table IV). In FW, plasma
301 osmolality was 4.9% higher in the L♀R♂ cross-type than in the L♀L♂ fish (Fig. 3a). Swimming to
302 exhaustion in SW was associated with an increase in plasma osmolality in all groups of fish, but plasma
303 osmolality was 6% higher in resident fish than in the two hybrid cross-types (Table IV; Fig. 3a).
304 Na^+K^+ATPase capacity was similar among cross-types that swam in FW (significant interaction
305 between factors with no significant covariance effect; Table IV), but activity was almost three times
306 higher in R♀R♂ individuals than in the other three cross-types in SW challenges (Fig. 3b).

307

308 Blood haematocrit varied according to cross-type (Table IV) and was positively correlated to body
309 mass. Blood haematocrit was 12% lower in L♀L♂ fish (the smallest cross-type) than in the other cross-
310 types (Fig. 4a). Blood haemoglobin varied according to both cross-type and salinity (significant
311 interaction between factors), and a significant positive body mass covariance effect was noted (Table
312 IV). In SW, blood haemoglobin concentration was highest in L♀R♂ hybrids while no difference could

313 be seen among cross-types in fish that swam in FW (Fig. 4b). The resulting MCHC differed among
314 cross-types but not salinities: there was no significant covariate effect for body mass (Table IV).
315 MCHC was 16% lower in R♀L♂ than in L♀R♂ hybrids, and MCHC levels in hybrids were similar to
316 their respective maternal line (Fig. 4c).
317
318 ENERGY RESERVES
319
320 A significant interaction between cross-type and salinity was observed for muscle glycogen content
321 with no body mass covariance effect (Table IV). After fish were challenged in FW, muscle glycogen
322 content was 64.4% lower in anadromous and R♀L♂ hybrids than in R♀R♂ fish (Fig. 5a). The muscle
323 glycogen content in the other hybrid was intermediate to those of the parental lines. Following
324 exhaustion in SW, muscle glycogen content was similar among cross-types (Fig. 5). Within each cross-
325 type, muscle glycogen content was similar whether swimming challenges were performed in FW or
326 SW. A significant interaction between cross-type and salinity was also observed for liver glycogen
327 content along with a significant positive body mass covariance effect (Table IV). Exhaustion in SW or
328 FW only had a distinct effect in L♀R♂ hybrids, for which liver glycogen was 60% lower after the SW
329 challenge compared to the concentration in fish exercised in FW (Fig. 5b). In FW-exhausted fish, liver
330 glycogen was ~ 60% lower in Laval fish than in the three other cross-types, while liver glycogen
331 concentration in SW was 56% lower in L♀L♂ and L♀R♂ than in the two other cross-types.
332
333 METABOLIC RESPONSE
334
335 There was a significant interaction between cross-type and salinity for white muscle CS capacity
336 (Table IV). In FW, CS capacity was 27% higher in the Rupert fish (R♀R♂) than in the other cross-
337 types, while no cross-type difference was observed in SW-exhausted fish (Fig. 6a). No salinity effect

was present within cross-types. CS K_m was also similar between fish challenged in FW (0.012 mM l^{-1}) and SW (0.011 mM l^{-1}). White muscle LDH capacity varied with both cross-type and salinity (but without significant interaction), and a significant positive body mass covariance effect was present (Table IV). The LDH capacity was 48% lower in L♀L♂ fish than in the three other cross-types (Fig. 6b), and LDH K_m was similar for fish swim-challenged in FW (0.79 mM l^{-1}) and SW (1.00 mM l^{-1}).

Muscle lactate concentration was different among cross-types and salinity trials (Table IV), and there was a positive correlation with body mass (Table IV). The L♀L♂ fish had 66% less muscle lactate compared to the R♀R♂ and L♀R♂ cross-types while the concentration in R♀L♂ hybrids was intermediate (Fig. 7a). Within each cross-type, no difference was present between swimming trials in FW or SW. A significant interaction between cross-type and salinity was observed for muscle pyruvate content along with a significant negative correlation with body mass (Table IV). After the FW challenge, muscle pyruvate content in L♀R♂ hybrids was 3.7 times lower than in the R♀R♂ cross-type (Fig. 7b), but there was no difference among cross-types following exhaustion in SW. Within cross-types, only L♀R♂ hybrids exhibited a significant difference in muscle lactate between FW and SW challenges: the muscle lactate/pyruvate ratio was 2.7 times higher in FW compared to SW (Fig. 7c), and a significant negative body mass covariance effect was observed (Table IV).

There was a significant interaction between cross-type and salinity on heart lactate content with a concomitant negative body mass covariance effect (Table IV). After challenge in FW, the heart lactate concentration of R♀L♂ hybrids was 37% lower than in purebred crosses (Fig. 7d) while it was highest in this cross-type following SW swimming exhaustion. Thus heart lactate concentration differed between the two environments only in the R♀L♂ cross-type (1.9 times higher in FW than in SW). Heart pyruvate concentration also varied according to cross-type and salinity (but without interaction), with a significant negative body mass covariance effect (Table IV): it was 69% higher in L♀L♂ fish than in

363 R♀L♂ hybrids. Globally, heart pyruvate concentration was 34.6% lower after the SW swimming
364 challenge than after the FW challenge (Fig. 7e). This resulted in the highest heart lactate/pyruvate ratio
365 for R♀L♂ hybrids challenged in SW (twice as high as the overall mean ratios of all other challenged
366 fish) (Fig. 7f).
367
368 **DISCUSSION**
369
370 The main objective of this study was to test for the occurrence of functional divergence in the factors
371 affecting swimming performance (estimated by U_{crit}) between pure strains and reciprocal hybrids
372 issued from two wild populations of *S. fontinalis* having different migratory lifestyles (Laval strain:
373 anadromous; Rupert strain: freshwater resident). Pure cross types had similar swimming performance
374 in FW, and swimming performance was reduced by 14% following abrupt transfer to SW in both
375 anadromous and resident fish. However, the pure cross types reached similar swimming speeds using
376 different physiological strategies, suggesting different genetically-based physiological solutions to the
377 same functional challenge. While no evidence was found for extreme non-additive genetic effects (i.e.
378 heterosis or outbreeding depression) in hybrids, significant differences between the two reciprocal
379 hybrids (L♀R♂ vs R♀L♂) were noted, with lower performance in L♀R♂.
380
381 PURE STRAINS
382
383 Fishes swimming performance is controlled by a number of physiological, morphological, and
384 behavioural traits, all of which interact and involve potential trade-offs (Walker, 2010; Dalziel *et al.*,
385 2011; Marras *et al.*, 2013). Considering the principle of many-to-one mapping, many different
386 combinations of traits can generate equivalent performance and multiple underlying factors can affect a
387 single quantitative trait (Wainwright *et al.*, 2005; Walker, 2010; Dalziel *et al.*, 2011).

Condition factor data are consistent with previous studies, which showed that anadromous fishes are more streamlined than resident fishes (Taylor & Foote, 1991; Eliassen *et al.*, 1998; Howland *et al.*, 2001; Morinville & Rasmussen, 2008; Dalziel *et al.*, 2011). On that basis, the similar swimming performance of resident and anadromous fish may seem counterintuitive as the most streamlined body shape of the anadromous strain should be energetically advantageous. Swimming is energetically demanding and requires high aerobic metabolic capacity (Gamperl *et al.*, 2002; Tudorache *et al.*, 2008; Dalziel *et al.*, 2011; Eliason & Farrell, 2016). Resident fish must then compensate for the advantage that body shape conferred to anadromous fish.

Here, the results suggest that anaerobic swimming contributed more to their overall swimming performance. In both FW and SW, maximal swimming was associated with a muscle lactate concentration and an LDH capacity that was twice as high in resident compared to anadromous fish, suggesting a larger contribution of anaerobic component in the former. Despite a 20% higher white muscle CS capacity in resident fish exercised in FW, no clear between-strain difference or pattern emerged regarding aerobic performance. It should be noted that CS activity was low in both resident and anadromous fish.

Higher glycogen storage and more efficient mobilization and utilization have been suggested to improve swimming performance (Fu *et al.*, 2011; Yang *et al.*, 2015). During anaerobic swimming, fishes white muscles rely on three endogenous fuel sources *i.e.*, adenosine triphosphate, phosphocreatine and glycogen. In the very first stages of white muscle mobilization, adenosine triphosphate and phosphocreatine stores are rapidly exhausted (Dobson and Hochachka, 1987) and it is glycogenolysis that then provides most of the ATP anaerobically, depleting muscle glycogen (Wood, 1991; Milligan, 1996). The Rupert fish (FW resident) may not only have reached a swimming

413 performance similar to that of anadromous fish due to their greater anaerobic capacities, but also
414 because of higher energy reserves. The glycogen levels in epaxial muscle and liver following FW
415 exercise were more than twice as high in resident than in anadromous fish. The exception was the
416 epaxial muscle of resident fish tested in SW, which may indicate greater energetic demand following
417 this trial. Thus the resident population compensated for its lower natural swimming ability (compared
418 to the anadromous population) by having a higher metabolic capacity.
419
420 For species moving between FW and SW, a large osmoregulatory capacity is an additional and
421 critically important determinant for maintaining swimming performance (Brauner *et al.*, 1992; Nelson
422 *et al.*, 1996; McKenzie *et al.*, 2001b; Chatelier *et al.*, 2005). Regardless of FW rearing conditions,
423 cross-type differences in the stress response to SW transfer were expected and a lower SW swimming
424 performance in resident fish. Following the SW challenge, resident fish had plasma osmolality similar
425 to anadromous fish combined with a gill Na^+-K^+-ATPase activity that was 4.4 times higher. However,
426 no differences in other stress indicators were observed whether fish were exercised in FW or in SW.
427 One may ask why experimental animals were reared in FW. In captivity, rearing 0+ and 1+ animals for
428 prolonged periods in SW greatly increased events of opportunistic myxobacteria infections, suggesting
429 impaired homeostasis, which is why young stages are routinely maintained in FW (C. Audet,
430 unpublished data). Otherwise, 2+ and older anadromous Laval fish (including breeders) are reared at a
431 salinity of 20 between the beginning of June and late September, mimicking the migration pattern of
432 this wild anadromous fish population (Curry et al. 2010).
433
434 Previous studies comparing the performance of anadromous and resident populations in different
435 fishes species showed that anadromous fishes possessed significantly greater swimming capacities than
436 those from resident populations (*O. kysutch*: Taylor & Foote, 1991; *S. fontinalis*, *Salmo trutta*, *S. salar*:
437 Peake *et al.*, 1997; *G. aculeatus*: Dalziel *et al.*, 2011; Kitano *et al.*, 2012). It has been hypothesized that

438 their exposure to fast-water habitats, which are more energetically costly, allowed the anadromous
439 fishes to evolve more efficient swimming abilities than resident populations (*O. kysutch* Taylor &
440 Foote, 1991; *S. fontinalis, S. trutta, S. salar*, Peake *et al.*, 1997; *S. fontinalis*: Morinville & Rasmussen,
441 2003; 2008). In the present study, even though the swimming performance was similar between
442 anadromous and freshwater resident fish, the results indicate a higher contribution of non-aerobic
443 pathways in resident fish which suggests that they may be less adapted to sustained swimming.
444
445 RECIPROCAL HYBRIDS
446
447 Swimming performance and its underlying traits were different between the reciprocal hybrids.
448 Compared to R♀L♂ hybrids, L♀R♂ hybrids had a 20% lower swimming speed, which was associated
449 with a 24% smaller cardio-somatic index, a 21% higher MCHC, and a 19% higher haemoglobin
450 concentration when swimming in SW as well as a larger metabolic (1.9 times higher muscle lactate
451 accumulation) and energetic (44% less liver glycogen in SW) response. L♀R♂ hybrids thus expended
452 greater effort and still had a lower performance than the reciprocal hybrid. Therefore, this performance
453 depends on cross direction (parental line used as dam or sire). Such cross-direction phenomena have
454 also been reported in *M. salmoides* (Cooke *et al.*, 2001) and Chinook salmon *Oncorhynchus*
455 *tshawytscha* (Walbaum 1792) (Falica & Higgs, 2012), but hybrids can often be similar in their
456 swimming performance (Hawkins & Quinn, 1996; Dalziel *et al.*, 2011). The reciprocal effect may be
457 explained by various factors such as maternal or paternal effects, or genetic linkage between sex genes
458 and performance genes. Swimming performance may be influenced by maternal effects, which are
459 often involved in cross direction. However, these effects generally occur during early life development
460 (due to egg size or yolk quality) with a decrease over time, and thus should probably be negligible in
461 the present study since fish were tested at age 1+ (Taylor & Foote, 1991; Heath *et al.*, 1999; Perry *et*
462 *al.*, 2004; Perry *et al.*, 2005). Paternal effect could have a strong influence on swimming performance;

this was the explanation given for the cross direction observed in *M. salmoides* and *O. tshawytscha*. The underlying genetic mechanisms of these sire effects still need to be more thoroughly investigated (Cooke *et al.*, 2001; Evans *et al.*, 2004; Falica & Higgs, 2012), but could hypothetically be under genetic control. In the present study, no evidence of paternal effect was found. The genetic linkage between sex genes and genes associated with performance traits can result in sex-specific gene expression under the control of the sex-determining region (Ellegren & Parsch, 2007; Derome *et al.*, 2008), which might then influence the predominance of a specific parental line as dam or sire in the expression of performance. Testing this hypothesis will require further investigation. In addition, other possible effects related to the genetic architecture (*e.g.*, pleitropy or other genetic linkage) of swimming performance merit further investigation.

GENETIC AND EVOLUTIONARY CONSIDERATIONS

Because the experiment was conducted in a common garden environment, differences in condition factor and physiological support features must have a genetic basis specific to each population. The different underlying traits affecting swimming performance thus have the potential to evolve under natural selection as does swimming performance itself, for which heritability has recently been estimated in European sea bass *D. labrax* (Vandeputte *et al.*, 2016). Similar results have been observed between different populations of Atlantic cod *Gadus morhua* (L. 1758) originating from different salinity environments (salt and brackish water) and tested in both environments (Nelson *et al.*, 1996). In the Nelson *et al*. (1996) study, swimming performance (U_{crit}) did not differ between populations even though there were inter-population differences in key support performance traits such as metabolic rate and aerobic and anaerobic capacities. These populations had been separated for less than 3000 years, and the authors considered that this was too short for genetic changes to have occurred under normal natural selection; they rather suggested that these inter-population differences mostly

488 resulted from acclimation. More recent studies have suggested that genetic adaptation could occur very
489 quickly, *e.g.*, within a small number of generations (Reznick *et al.*, 1997; Pearse *et al.*, 2009; Ellner *et*
490 *al.*, 2011; Westley *et al.*, 2013). Since the separation of the *S. fontinalis* populations used in this study
491 occurred around 10 000 years ago (Castric & Bernatchez, 2003), it seems that such a time frame would
492 have been sufficient for the different populations to evolve distinct genetically based physiological
493 adaptations to cope with their respective environments.
494
495 Differences between the two populations could be the results of local adaptation to different
496 migratory lifestyles. Since swimming performance integrates the actions of a large number of organs
497 and supporting functions, the investigation of the variability in swimming capacity within and among
498 populations can be considered as a relevant means to reveal elements of local adaptation (Cooke *et al.*,
499 2001; Odell *et al.*, 2003; Pon *et al.*, 2007). Although this needs to be more rigorously investigated,
500 ecological differences in the populations' migratory conditions (*i.e.*, differences in fluctuations of
501 temperature, velocity, and salinity experienced by the anadromous and the resident populations in their
502 respective environments) could have influenced the physiological processes involved in swimming
503 performance. Since the resident population likely faces strong currents during spring, swimming ability
504 probably remained a key determinant of fitness for freshwater residency. However, it should be noted
505 that the crosses in this study were only between the Rupert and the Laval strains. It is possible that
506 crosses involving different anadromous and resident *S. fontinalis* populations could lead to results
507 different from what was found here. Thus the possibility exists that the differences observed between
508 the Rupert and Laval strains might not be linked to their migratory behaviour but to other forces
509 shaping local adaptation. The Rupert and Laval fish used for this study were F3 fish, and domestication
510 effects may already be present (Sauvage *et al.*, 2010). However, other studies done on the same
511 families have shown that they are still very different in terms of reproductive period, stress response
512 (Crespel *et al.*, 2011), growth, gene × environment interactions on growth (Crespel *et al.*, 2013a), and

storage and use of energy reserves (Crespel *et al.*, 2013b). Could short-term domestication have eliminated differences in swimming capacity but maintained differences in other traits? It is a possibility that cannot be completely rejected.

One of the objectives was to test the occurrence of non-additive effects in the hybrids. No evidence of heterosis or outbreeding depression was observed. When populations are very divergent and adapted to their respective environments, this may provide evidence that their genome has evolved towards local genetic complex associations. Hybridization between divergent populations alter these associations, and hybrids may thus express extreme non-additive genetic effects that can be positive (when hybrids outperform parental lines due to synergy between the genomes: heterosis) or negative (when hybrids underperform parental lines due to incompatibilities between the genomes: outbreeding depression) (Edmands, 1999; Cooke *et al.*, 2001; Stelkens *et al.*, 2009). Outbreeding depression has been observed in *M. salmoides* for the swimming performance of hybrids between two locally adapted populations, revealing a breakdown of co-adapted gene complexes (Cooke *et al.*, 2001; Cooke & Philipp, 2005; 2006). In the present study, which used two populations with different migratory lifestyles known to have very divergent genetic bases from both neutral (Martin *et al.*, 1997) and functional (Bougas *et al.*, 2010) standpoints, the occurrence of extreme non-additive genetic effects—and most specifically, outbreeding depression—would be expected (Bieri & Kawecki, 2003; Cooke & Philipp, 2005). However, this was not the case. The absence of pronounced non-additive effects for swimming and the underlying performance between the two populations that was found thus suggest that the extent of the genetic differences that have accumulated between these populations since their separation has not been sufficient to cause genomic incompatibilities between the parental genomes (Bieri & Kawecki, 2003; Rosenfield *et al.*, 2004) .

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808
809

Table I: Summary of experimental design: experimental groups of *S. fontinalis* used to test the repeatability of the swimming tests and perform the critical swimming speed (U_{crit}) test in salt water (SW) and the control groups with different fish used to perform the critical swimming speed (U_{crit}) test in fresh water (FW). L: Laval strain; R: Rupert strain.

	L♀L♂	L♀R♂	R♀L♂	R♀R♂
Experimental group				
Repeatability test 1 (FW)	$n = 2 \times 15$	$n = 2 \times 15$	$n = 2 \times 15$	$n = 2 \times 15$
Repeatability test 2 (FW)	$n = 2 \times 15$	$n = 2 \times 15$	$n = 2 \times 15$	$n = 2 \times 15$
Repeatability test 3 (FW)	$n = 2 \times 15$	$n = 2 \times 15$	$n = 2 \times 15$	$n = 2 \times 15$
U_{crit} (SW)	$n = 2 \times 15$	$n = 2 \times 15$	$n = 2 \times 15$	$n = 2 \times 15$
Control group				
U_{crit} (FW)	$n = 1 \times 10$	$n = 1 \times 10$	$n = 1 \times 10$	$n = 1 \times 10$

Table II: Repeatability of critical swimming speed (U_{crit}, L_S s^{-1}) in the two purebred strains of *S. fontinalis* (L♀L♂ and R♀R♂) and their reciprocal hybrids (L♀R♂ and R♀L♂). The repeatability tests were done in fresh water. Mean ± SE. U_{crit} among trials were not statistically different.

	L♀L♂	L♀R♂	R♀L♂	R♀R♂
n	30	30	30	30
U_{crit} 1	2.85 ± 0.21	2.83 ± 0.20	3.08 ± 0.13	2.24 ± 0.11
U_{crit} 2	2.59 ± 0.18	2.65 ± 0.17	3.00 ± 0.17	1.90 ± 0.11
U_{crit} 3	2.22 ± 0.15	2.47 ± 0.10	3.13 ± 0.18	2.44 ± 0.11

n = the number of individuals per swim test

Table III: Morphological characteristics (standard length [L_S], body mass [M_B], condition factor [CF], and cardio-somatic index [I_C]) of the two purebred strains of *S. fontinalis* (L♀L♂ and R♀R♂) and their reciprocal hybrids (L♀R♂ and R♀L♂) used for swimming challenges and biochemical samples. Mean ± SE. Different letters indicate significant differences among cross-types ($\alpha = 0.05$).

	L♀L♂	L♀R♂	R♀L♂	R♀R♂
n	38	40	40	38
L_S(cm)	11.08 ± 0.16^{a}	13.29 ± 0.34^{c}	12.00 ± 0.24^{b}	11.94 ± 0.21^{b}
M_B (g)	11.11 ± 0.61^{a}	21.98 ± 1.98^{c}	13.63 ± 0.91^{a}	17.30 ± 0.95^{b}
CF(g cm^{-3})	0.79 ± 0.02^{a}	0.86 ± 0.02^{b}	0.76 ± 0.03^{a}	0.98 ± 0.02^{c}
I_C(%)	0.15 ± 0.01^{ab}	0.14 ± 0.01^{a}	0.18 ± 0.01^{b}	0.16 ± 0.01^{ab}

n = the number of individuals

Table IV

Table IV: Summary of ANOVA results for the different variables measured in *S. fontinalis*: swimming challenge (critical swimming speed [U_{crit}]), stress and osmotic response (cortisol, glucose, muscle water, osmolarity, gill Na^+K^+ATPase, haematocrit, haemoglobin, mean cellular haemoglobin concentration [MCHC]), energy reserves (muscle glycogen, liver glycogen), metabolic response (citrate synthase [CS], lactate dehydrogenase [LDH], muscle lactate, muscle pyruvate, muscle lactate/pyruvate ratio [muscle ratio L/P], heart lactate, heart pyruvate, heart lactate/pyruvate ratio [heart ratio L/P]). Significant results are in bold. The variables for which body mass (covariable) had a significant effect are indicated with grey shading. When body mass had no significant effect, two-way ANOVAs were performed.

	Cross-type effect			Salinity effect			Cross-type ×Salinity			Body mass covariable			
	F	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	r^2
U_{crit}	**2.86**	**3**	**0.04**	**11.85**	**1**	**<0.01**	0.52	3	0.67				
Cortisol	1.19	3	0.32	0.09	1	0.77	0.16	3	0.92				
Glucose	**5.62**	**3**	**<0.01**	1.13	1	0.3	**2.9**	**3**	**0.04**				
Muscle water	2.12	3	0.1	**33.9**	**1**	**<0.01**	1.77	3	0.16	**4.86**	**1**	**0.03**	**-0.17**
Osmolarity	**5.1**	**3**	**<0.01**	**96.35**	**1**	**<0.01**	**5.69**	**3**	**<0.01**	**12.31**	**1**	**<0.01**	**-0.26**
Gill Na^+K^+ATPase	**9.78**	**3**	**<0.01**	0.91	1	0.34	**3.76**	**3**	**0.01**				
Haematocrit	**4.6**	**3**	**<0.01**	3.51	1	0.06	2.08	3	0.11	**14**	**1**	**<0.01**	**0.36**
Haemoglobin	0.81	3	0.49	2.51	1	0.11	**3.42**	**3**	**0.02**	**8.15**	**1**	**<0.01**	**0.29**

MCHC	**5.11**	**3**	**<0.01**	6.04	1	0.02	2.03	3	0.11				
Muscle glycogen	**5.47**	**3**	**<0.01**	**5.23**	**1**	**0.02**	**4.13**	**3**	**<0.01**				
Liver glycogen	**14.27**	**3**	**<0.01**	**9.94**	**1**	**<0.01**	**5.57**	**3**	**<0.01**	**4.05**	**1**	**0.05**	**0.31**
CS	**11.11**	**3**	**<0.01**	**10.14**	**1**	**<0.01**	**4.79**	**3**	**<0.01**				
LDH	**16.44**	**3**	**<0.01**	**5.59**	**1**	**0.02**	0.36	3	0.78	**118.76**	**1**	**<0.01**	**0.67**
Muscle lactate	**14.5**	**3**	**<0.01**	0.13	1	0.72	**3.85**	**3**	**0.01**	**46.02**	**1**	**<0.01**	**0.61**
Muscle pyruvate	0.51	3	0.67	2.52	1	0.11	**3.77**	**3**	**0.01**	**20.97**	**1**	**<0.01**	**-0.44**
Muscle ratio L/P	2.25	3	0.08	**4.88**	**1**	**0.03**	**2.62**	**3**	**0.05**	**33.1**	**1**	**<0.01**	**0.56**
Heart lactate	0.23	3	0.87	2.04	1	0.16	**13.33**	**3**	**<0.01**	**4.9**	**1**	**0.03**	**-0.24**
Heart pyruvate	**6.07**	**3**	**<0.01**	**40.33**	**1**	**<0.01**	0.94	3	0.42	**43.28**	**1**	**<0.01**	**-0.38**
Heart ratio L/P	**6.06**	**3**	**<0.01**	**55.49**	**1**	**<0.01**	**8.26**	**3**	**<0.01**	**59.38**	**1**	**<0.01**	**0.32**

Figure captions

Figure 1: Schematic diagram of the cross-types used to test swimming performance in purebred crosses of *S. fontinalis* (bold) and of their reciprocal hybrids. Arrows with dashed lines (⇢) represent the various families (*n*=10) within cross-types and arrows with double lines (⟹) represent the number of fish sampled (*n*=100) from the different families. L: Laval strain; R: Rupert strain.

Figure 2: Critical swimming speeds of the two purebred strains of *S. fontinalis* and their reciprocal hybrids in fresh (black bars) and salt (white bars) water. The first letter of the cross-type indicates the dam and the second letter the sire. L: Laval strain; R: Rupert strain. Mean ± SE. Different letters indicate significantly different means among cross-types ($\alpha = 0.05$). Swimming speeds were generally higher in FW than in SW. No significant interaction between cross-type and salinity was found.

Figure 3: (a) Plasma osmolality and (b) gill Na^+-K^+ATP-ase specific activity in two purebred strains of *S. fontinalis* and their reciprocal hybrids following swimming trials in fresh (black bars) or salt (white bars) water. The first letter of the cross-type indicates the dam and the second letter the sire. L: Laval strain; R: Rupert strain. Mean ± SE. Different letters indicate significantly different means ($\alpha = 0.05$).

Figure 4: (a) Haematocrit, (b) blood haemoglobin, and (c) mean cellular haemoglobin concentration (MCHC) in two purebred strains of *S. fontinalis* and their reciprocal hybrids following swimming trials in fresh (black bars) or salt (white bars)water, or combined FW and SW data (grey bars). The first letter of the cross-type indicates the dam and the second letter the sire. L: Laval strain; R: Rupert strain. Mean ± SE. Different letters indicate significantly different means ($\alpha = 0.05$).

Figure 5: (a) Muscle and (b) liver glycogen concentrationin two purebred strains of *S. fontinalis* and their reciprocal hybrids following swimming trials in fresh (black bars) or salt (white bars) water.The first letter of the cross-type indicates the dam and the second letter the sire. L: Laval strain; R: Rupert strain. Mean ± SE. Different letters indicate significantly different means ($\alpha = 0.05$).

Figure 6: (a) Citrate synthase and (b) lactate dehydrogenase capacity in two purebred strains of *S. fontinalis* and their reciprocal hybrids following swimming trials in fresh (black bars) or salt (white bars) water, or combined FW and SW data (grey bars).The first letter of the cross-type indicates the dam and the second letter the sire. L: Laval strain; R: Rupert strain. Mean ± SE. Different letters indicate significantly different means ($\alpha = 0.05$).

Figure 7: (a) Muscle lactate, (b) muscle pyruvate, (c) muscle lactate/pyruvate ratio, (d) heart lactate, (e) heart pyruvate, and (f) heart lactate/pyruvate ratio in two purebred strains of *S. fontinalis* and their reciprocal hybrids following swimming trials in fresh (black bars) or salt (white bars) water. The first letter of the cross-type indicates the dam and the second letter the sire. L: Laval strain; R: Rupert strain. Different letters indicate significantly different means ($\alpha = 0.05$). Data are expressed as mean ± SE except for the muscle pyruvate concentration, for which solid squares, rectangles, and whiskers indicaterespectively the median, the middle two quartiles, and the range. Muscle pyruvate concentration data were not normally distributed and statistical analyses were done on ranks. However, to facilitate comparisons with other studies, data are presented using median and range. The muscle lactate/pyruvate ratio data were square-root transformed prior to statistical analysis. To facilitate comparisons with other studies, arithmetic data are presented.

Figure1

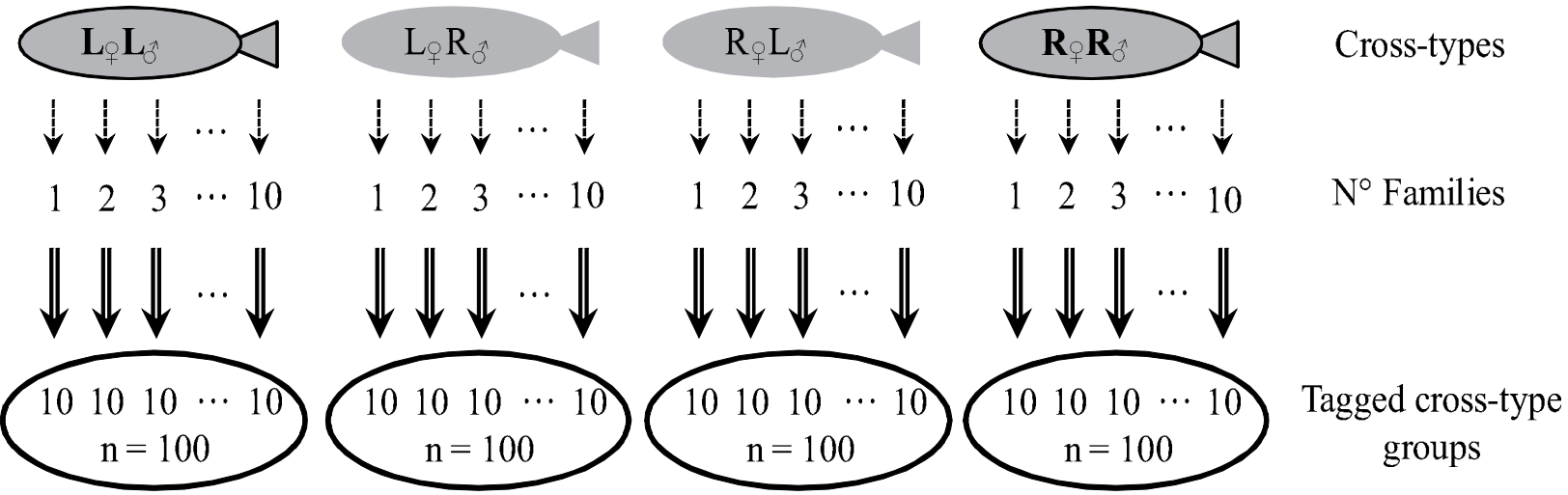
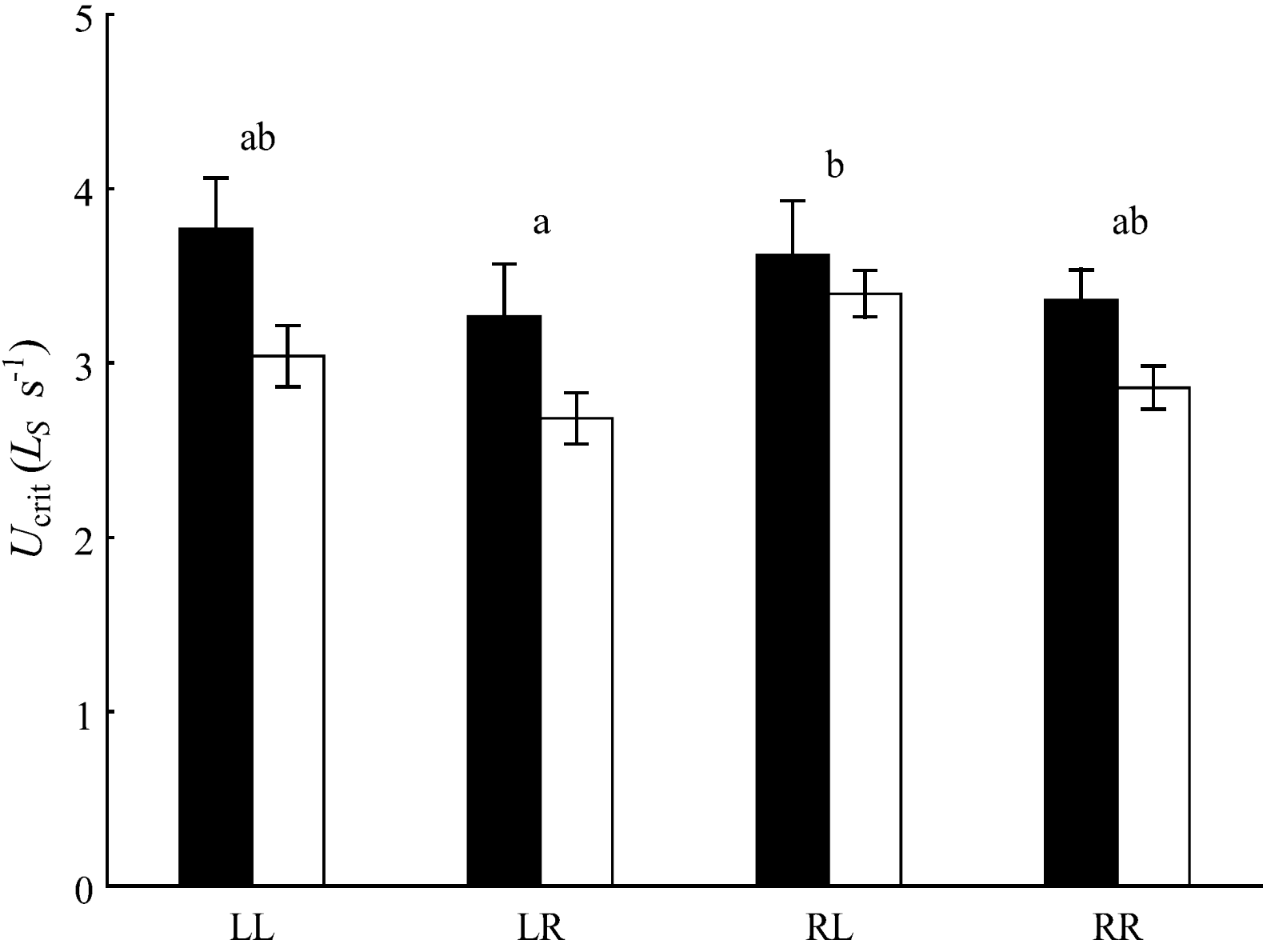

L♀L♂
L♀R♂
R♀L♂
R♀R♂
Cross-types
1 2 3 ··· 10
N° Families
10 10 10 ··· 10
n = 100
Tagged cross-type groups

Figure2



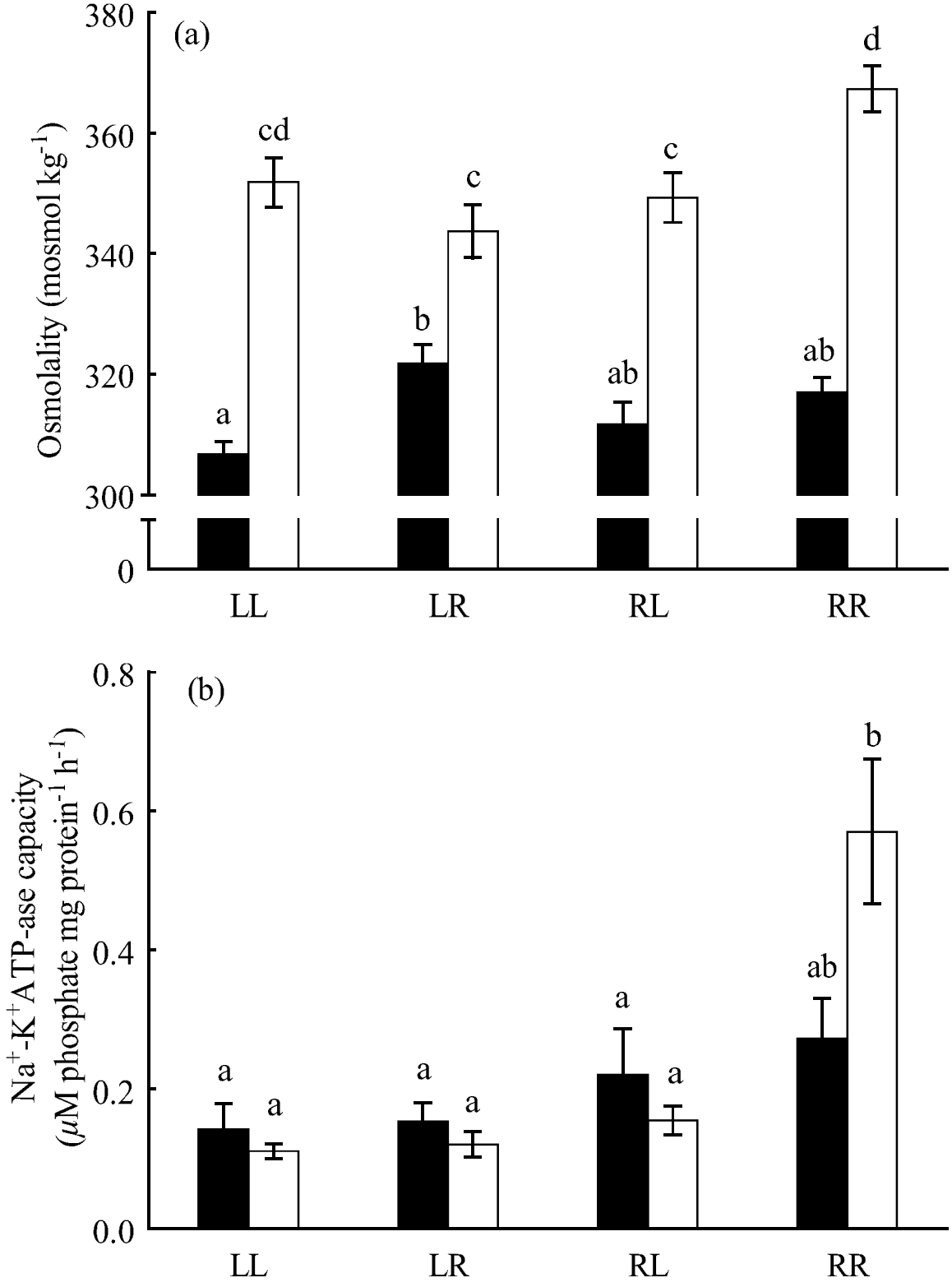

(a)
Osmolality (mosmol kg-1)
380
360
340
320
300
0
a
cd
b
c
ab
c
ab
d
LL
LR
RL
RR
(b)
Na+-K+ATP-ase capacity
(μM phosphate mg protein-1 h-1)
0.8
0.6
0.4
0.2
0.0
a
a
a
a
a
a
ab
b
LL
LR
RL
RR

Figure3

Figure4

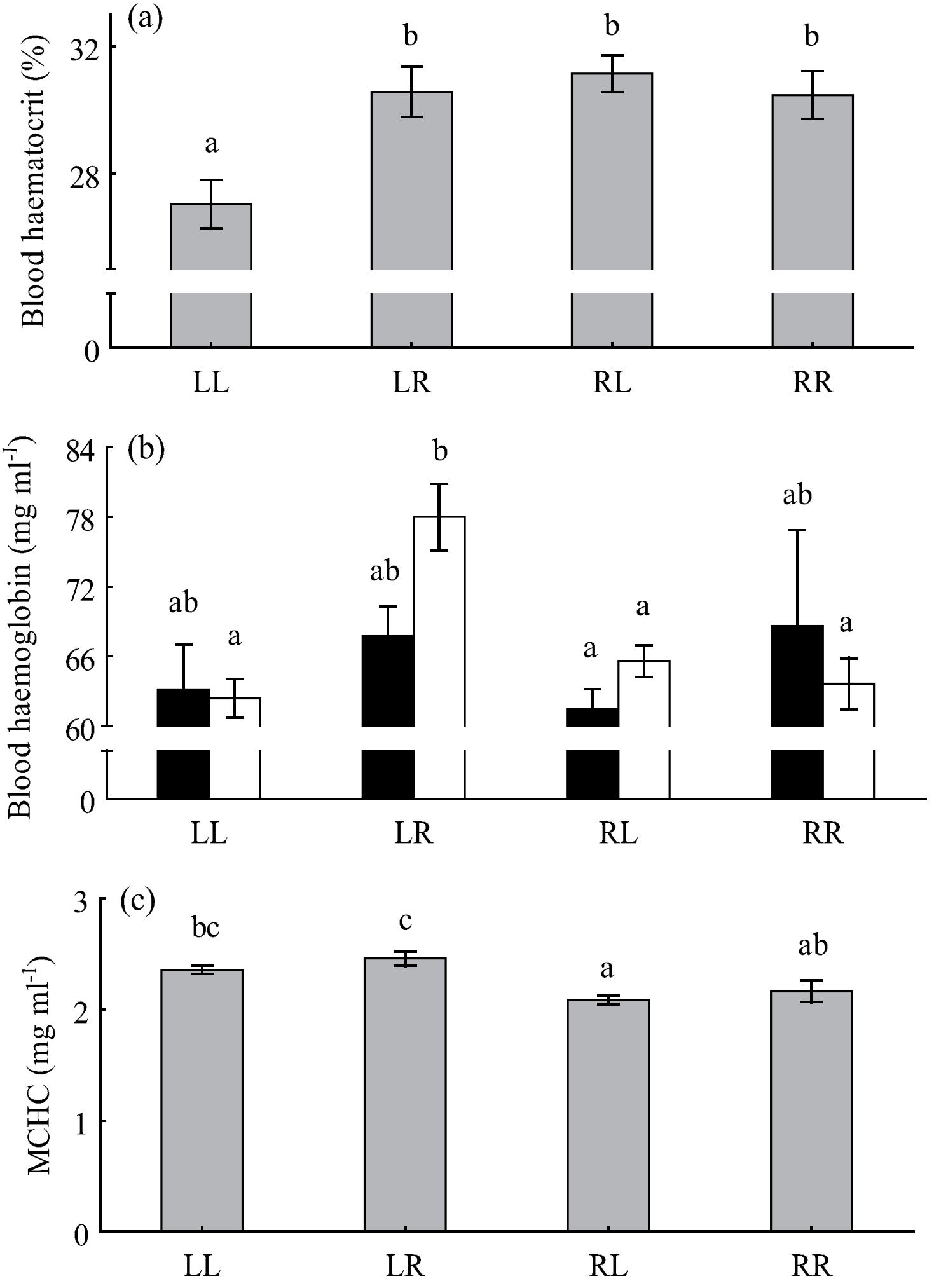

(a)
Blood haematocrit (%)
32
28
0
a
b
b
b
LL
LR
RL
RR
(b)
Blood haemoglobin (mg ml-1)
84
78
72
66
60
0
ab
a
ab
b
a
a
ab
a
LL
LR
RL
RR
(c)
MCHC (mg ml-1)
3
2
1
0
bc
c
a
ab
LL
LR
RL
RR

Figure5

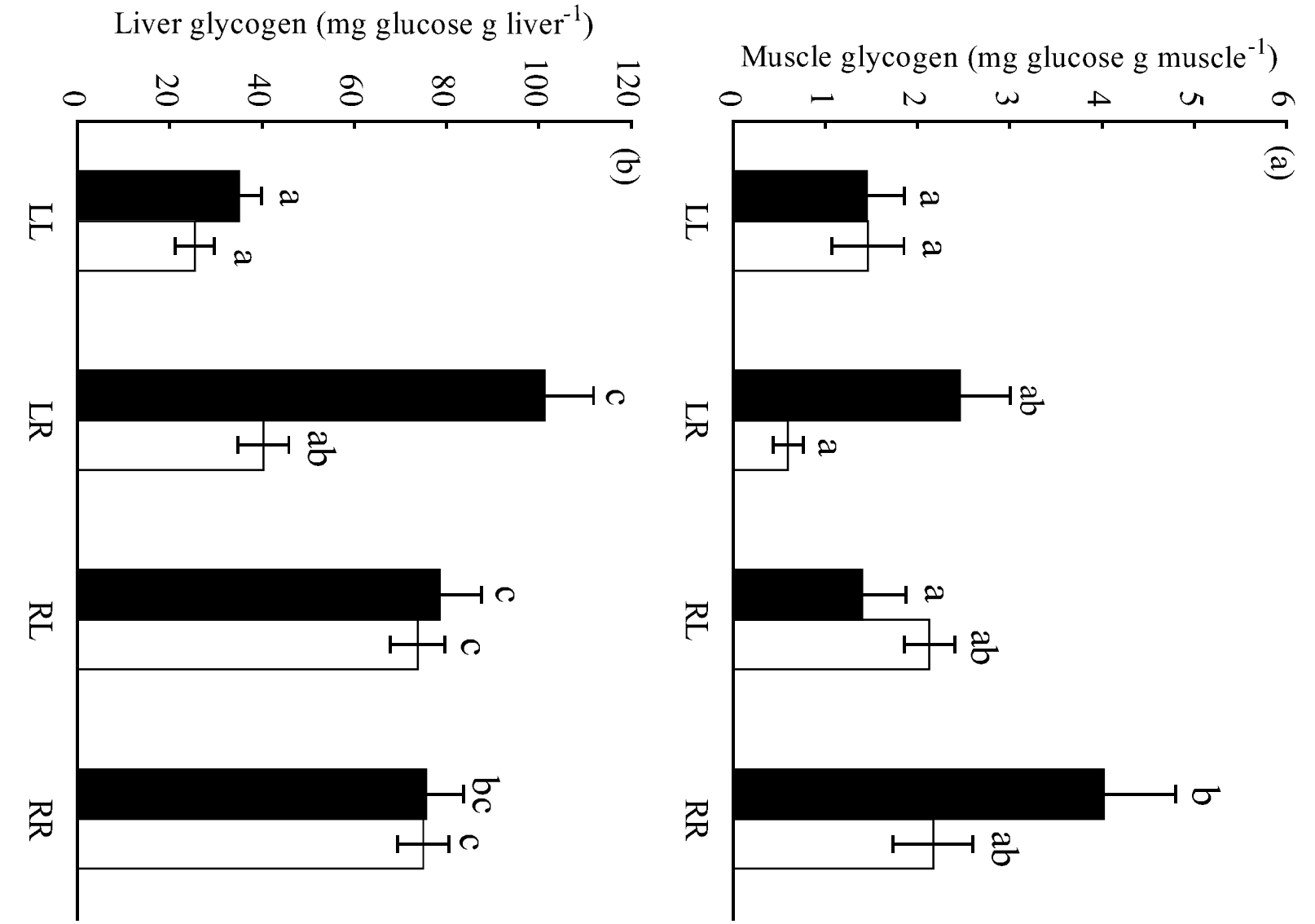
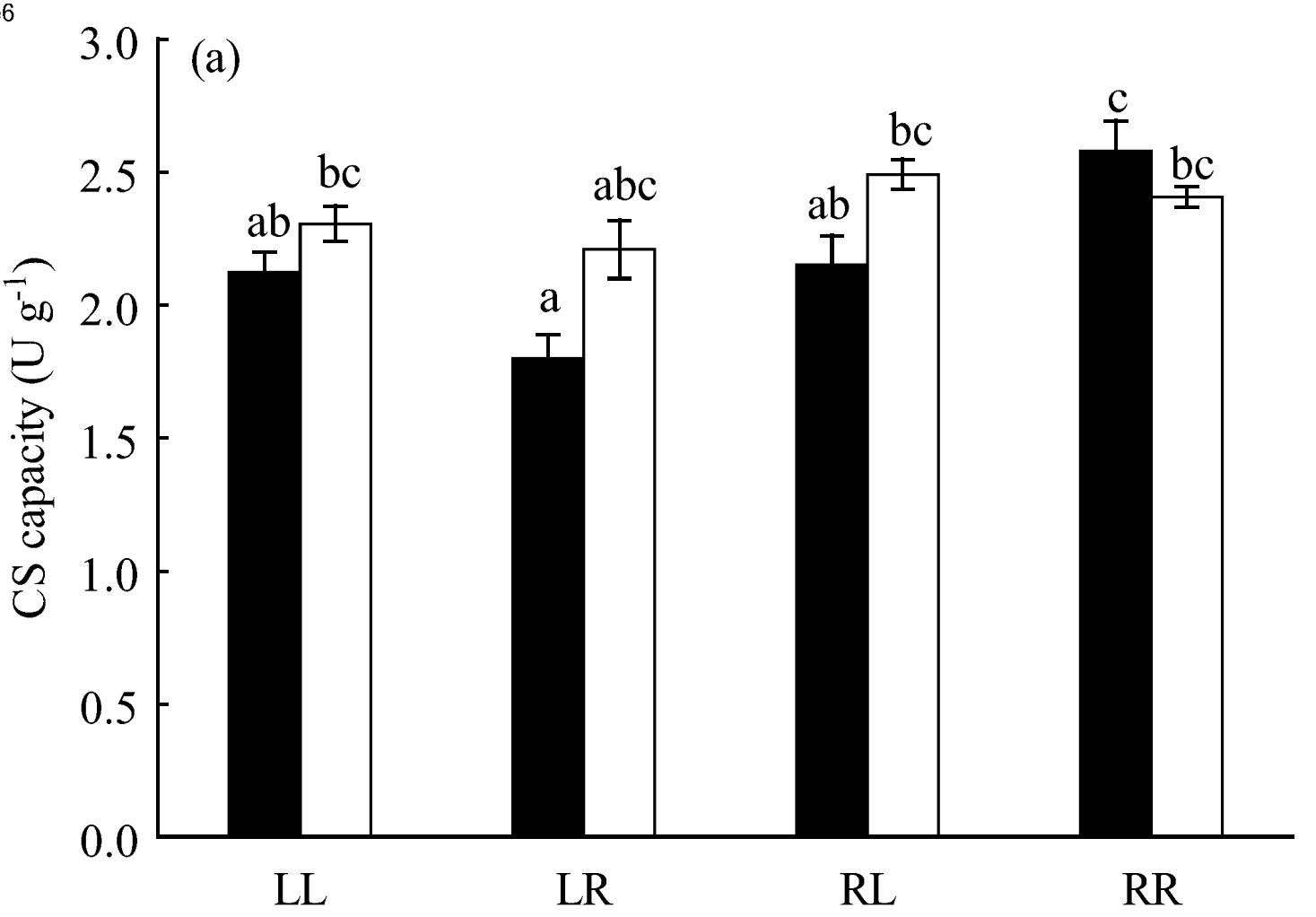

Muscle glycogen (mg glucose g muscle-1)
0
1
2
3
4
5
6
(a)
LL
LR
RL
RR
a
a
ab
a
a
ab
b
ab
Liver glycogen (mg glucose g liver-1)
0
20
40
60
80
100
120
(b)
LL
LR
RL
RR
a
a
c
ab
c
c
bc
c

Figure6


(a)
CS capacity (U g-1)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ab
bc
a
abc
ab
bc
c
bc
LL
LR
RL
RR

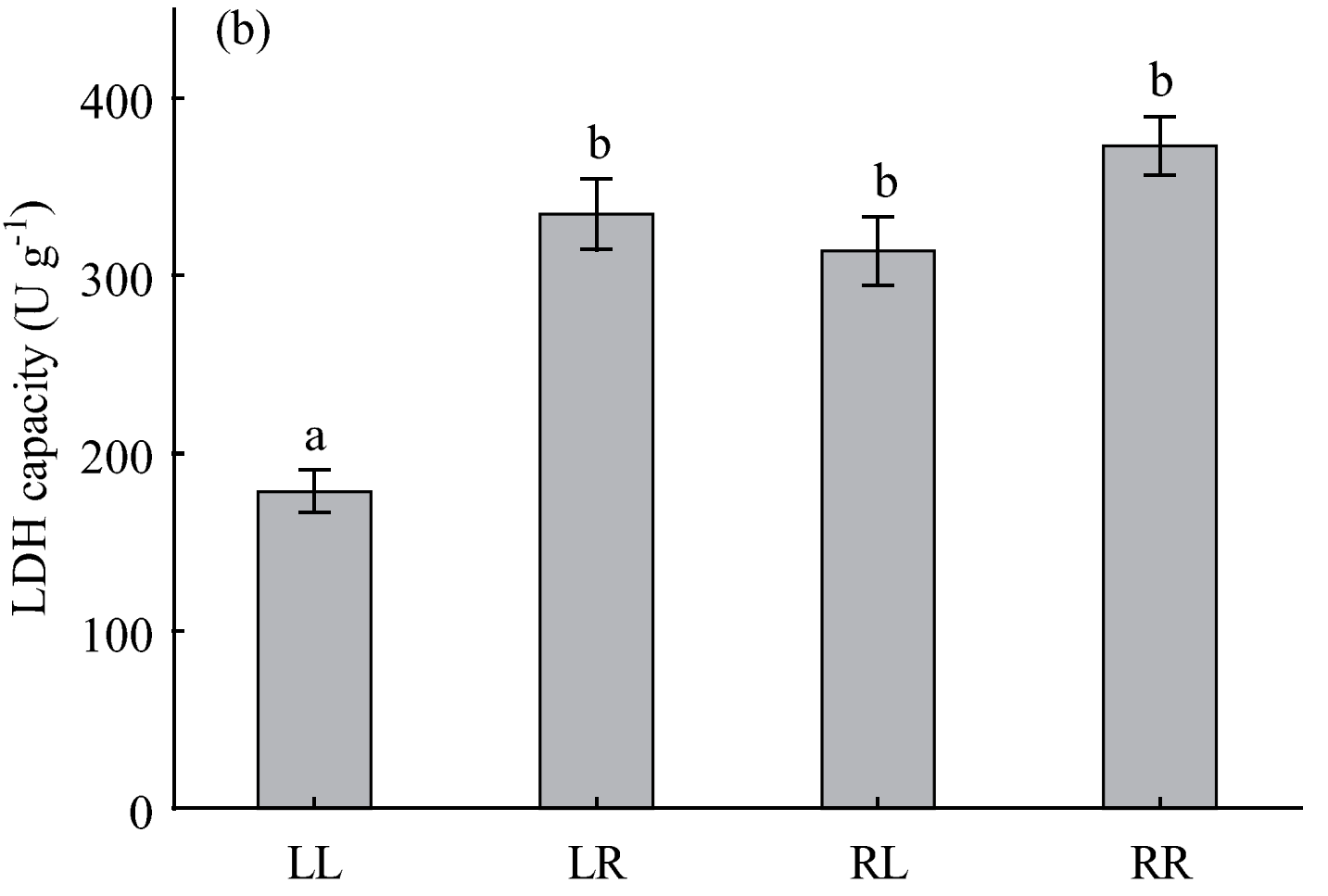
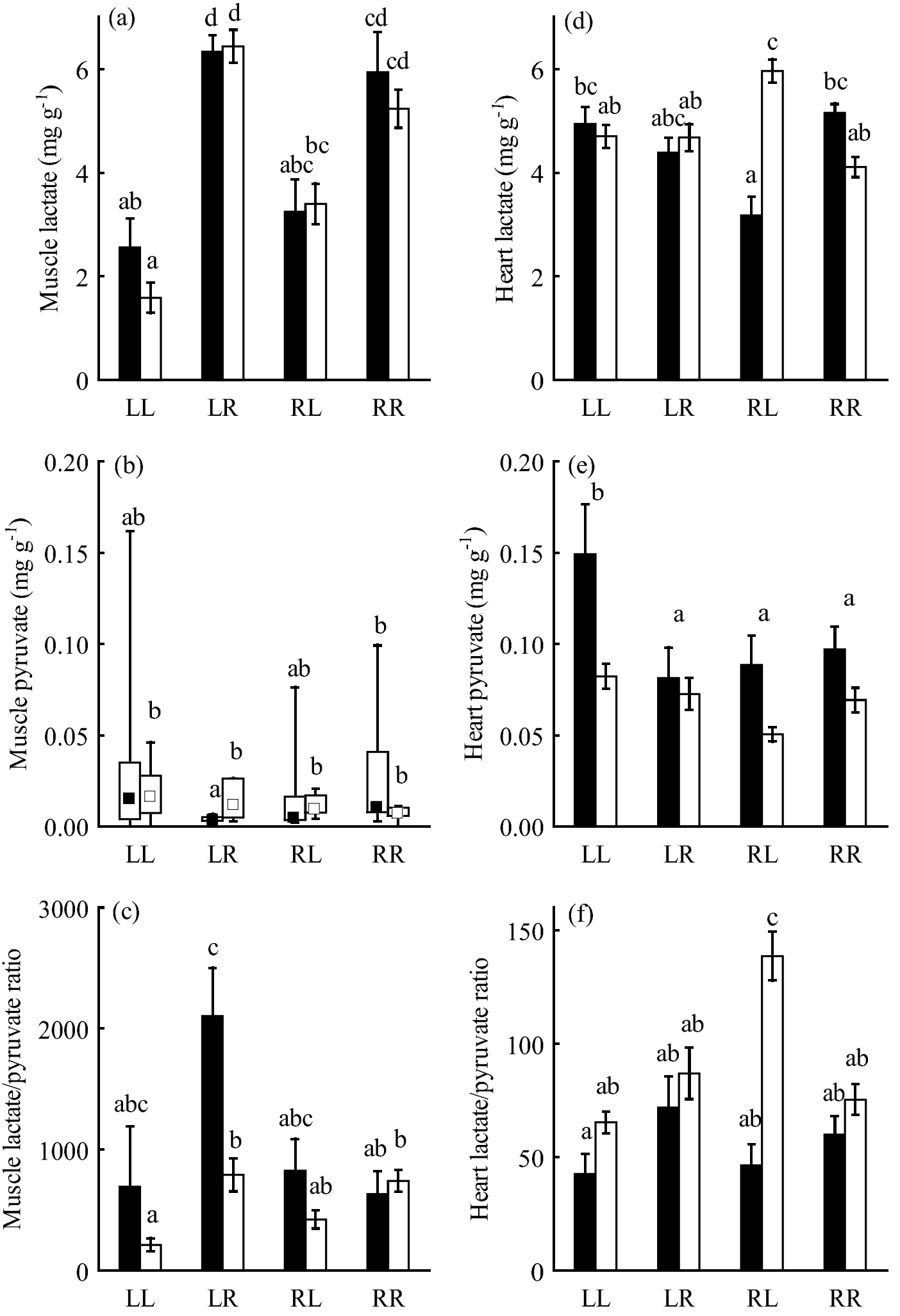

(b)
LDH capacity (U g-1)
400
300
200
100
0
a
b
b
b
LL
LR
RL
RR

Figure7


(a)
Muscle lactate (mg g-1)
0
2
4
6
ab
a
d
d
abc
bc
cd
cd
LL
LR
RL
RR
(d)
Heart lactate (mg g-1)
bc
ab
abc
ab
a
c
bc
ab
(b)
Muscle pyruvate (mg g-1)
0.00
0.05
0.10
0.15
0.20
ab
b
a
b
ab
b
b
b
(e)
Heart pyruvate (mg g-1)
b
a
a
a
(c)
Muscle lactate/pyruvate ratio
0
1000
2000
3000
abc
a
c
b
abc
ab
ab
b
(f)
Heart lactate/pyruvate ratio
0
50
100
150
a
ab
ab
ab
ab
c
ab
ab