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

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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 ($U_{\text{crit}}$) 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 $U_{\text{crit}}$ in both FW and SW, with $U_{\text{crit}}$ 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 $R_{\phi}L_{\delta}$ hybrids, $L_{\phi}R_{\delta}$ 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
species to maintain plasma osmolality and tissue water content when exposed to an acute change in ambient salinity (Chatelier *et al.*, 2005).

In salmonids, migratory behaviour has evolved as a mandatory phase in the life cycle of some species whereas it is optional in others (McDowall, 1997; Klemetsen *et al.*, 2003; Fraser & Bernatchez, 2005; Thériault *et al.*, 2007; Arai & Goto, 2008). In brook charr *Salvelinus fontinalis* Mitchill 1814, the ancestral form of anadromy is now facultative (Castric & Bernatchez, 2003; Curry *et al.*, 2010), and different migratory patterns exist depending on the biotic and abiotic conditions in the native environment of a population (Castric & Bernatchez, 2003). The anadromous *S. fontinalis* population of the Laval River (L) (48° 44' N; 69° 05' W) on the north shore of the St. Lawrence estuary migrates to freshwater for reproduction and overwintering and to salt water in summer for feeding. These fish can thrive in habitats encompassing a wide range of environmental conditions—from low to high salinity (1 to 34), temperature (5 to 18°C), and water velocities (Boula *et al.*, 2002; Curry *et al.*, 2006). The Rupert population (R) is a strictly freshwater resident *S. fontinalis* population originating from the Rupert River (51° 05' N; 73° 41' W) near Lake Nemiscau (near James Bay in NW Québec). These fish always live in cold freshwater and migrate from the river to lakes for reproduction (MAPA-Pêcheries, 1992). In addition to living in two different environments and having different lifestyles, previous genetic studies revealed a pronounced genetic differentiation between these two populations (Fst = 0.427 ± 0.020; Martin *et al.*, 1997), as well as important differences in gene expression when reared in a same environment (Bougas *et al.*, 2010). However, it is not known whether these differences are accompanied by a divergence in their swimming capacity.

Previous studies on salmonids have revealed that different lifestyles among species or populations may result in differences in their swimming ability (Taylor & McPhail, 1985; Hawkins & Quinn, 1996; Peake *et al.*, 1997). In Atlantic salmon *Salmo salar* (L. 1758), anadromous individuals possess greater
sustained swimming ability than landlocked ones, possibly related to their different morphology (the anadromous form has a more fusiform body shape than the landlocked one) and migratory histories (Peake et al., 1997). When swimming tests were conducted in common environments, the differences between populations remained (Taylor & Foote, 1991), suggesting a genetic basis for swimming performance and thus a potential for evolutionary adaptation. In threespine stickleback *Gasterosteus aculeatus* (L. 1758), comparisons of swimming performance in freshwater resident and anadromous populations, both in Europe and North America, have shown that anadromous fish had a greater swimming performance than the freshwater residents (Tudorache et al., 2007, European populations; Dalziel et al., 2011, North American populations). In the North American populations, this difference is genetically based (Dalziel et al., 2011). Understanding the genetic and physiological bases of evolutionary change in swimming capacity in *S. fontinalis* could provide further insight into the functional bases of differential adaptation in swimming capacity of fishes (Odell et al., 2003; Collin & Fumagalli, 2011; Dalziel et al., 2011).

Hybridization between different populations may also provide important information on the genetic basis of swimming performance and the degree of divergence between populations. For example, measuring traits in F1 hybrids could reveal the relative importance of additive or non-additive genetic effects in the expression of performance (Dalziel et al., 2011). When populations are genetically closer, hybrids tend to express additive genetic effects and show intermediate performance compared to their parental lines. On the contrary, when populations are genetically divergent and adapted to their own environments, hybrids may express non-additive genetic effects due to complex genetic associations (Falconer & Mackay, 1996; Edmands, 1999; Cooke et al., 2001; Cooke & Philipp, 2005; Stelkens et al., 2009). Non-additive genetic effects have been reported for various morphological and physiological traits such as size, survival, and other fitness-related traits in rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) (Tymchuk et al., 2009), *O. kisutch* (Emlen, 1991), and *S. fontinalis* (Granier et
al., 2011; Crespel et al., 2012), and also in swimming performance in largemouth bass Micropterus salmoides (Lacepède 1802) (Cooke et al., 2001). The occurrence of non-additive genetic effects controlling fitness-related traits thus provide further evidence for evolutionary divergence among the populations studied. However, the occurrence of non-additive genetic effects in swimming performance and its underlying physiological basis among populations with different migratory lifestyles has rarely been investigated.

Whether anadromous fish are better swimmers than freshwater residents has been tested, hypothesizing that this trait would be a major fitness component in migratory fish. In addition to condition factor and energy reserve levels, a whole range of physiological factors can affect fish swimming capacity, thus the measurement of these variables gives information on their relative contributions. Blood oxygen-carrying capacity was inferred from blood hematocrit and haemoglobin concentration, leading to the calculation of the mean cellular haemoglobin concentration. The capacities of experimental populations to mobilize energy reserves to fuel working muscles were compared by measuring blood glucose as well as liver and white muscle glycogen content. For the same reason, white muscle and heart pyruvate and lactate concentrations were also assessed. The activities of white muscle lactate dehydrogenase (LDH) and citrate synthase (CS) were measured because these enzymes are important regulators of aerobic and anaerobic metabolism responding to substrate/product ratios. These measurements provided insight into the relative contribution of aerobic vs. anaerobic pathways to meet the energy needs associated with swimming. Since the capacity to maintain plasma osmotic and ionic characteristics is a key factor affecting fish swimming capacity, gill Na+K+ATPase activity was also assessed.

The occurrence of non-additive effects in the hybrids was investigated to obtain additional insight 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
fish from each family (100 fish per cross-type) were tagged using fingerling tags (Floy Tag Inc., www.floytag.com) (Fig. 1).

THE FLUME

The swimming tests were performed using a circular flume (with a linear swimming section) designed to provide non-turbulent water flow (Redjah et al., 2010). Briefly, a variable-speed motor propelled the water at a constant velocity. Plastic honeycomb structures and deflectors were inserted in the circulation loop upstream from the swimming chamber (23 × 37 × 22.3 cm) to promote rectilinear flow and a uniform velocity profile. An acoustic Doppler velocimeter (type 16 MHz MicroADV, Sonteck, www.sontek.com) was used to calibrate water velocity to voltage output from the motor controller. The flume was supplied with fully aerated and thermoregulated (6.8 ± 0.3°C) water at a flow rate of 10 l min⁻¹.

VALIDATION TEST AND CRITICAL SWIMMING SPEED PROTOCOL

To validate the swimming challenge procedure, two subgroups of 15 tagged fish per cross-type were submitted to three consecutive swim tests in FW with a 4 h recovery period between tests 1 and 2 and a 16 h recovery period between tests 2 and 3, which is in line with the capacity of salmonids to fully recover from exhaustion (45 to 90 min; Jain et al., 1998; Lee et al., 2003; Tierney & Farrell, 2004). Cross-types were tested separately, with subgroups of fish swimming together in each trial (Table I). The repeatability of individual performances was confirmed (Table II, $P > 0.05$) as was the fish swimming performance ranking (data not shown; $P > 0.05$).

Following transfer into the swimming chamber, fish were left undisturbed for 30 min at a water
speed of 5.5 cm s\(^{-1}\) (\textit{i.e.}, 0.5 standard length s\(^{-1}\) \([L_S \, s^{-1}]\)). Following this acclimation period, fish were 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 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 considered to be fatigued when they were unable to remove themselves from the screen situated downstream from the swimming chamber. At that time, fish were removed from the swim chamber, identified (tag reading), and placed in their original rearing tank. The corresponding water velocity and time were recorded. The critical swimming speed \(U_{\text{crit}}, L_S \, s^{-1}\) was calculated according to Brett (1964)

\[ U_{\text{crit}} = \frac{U + (T / T_i \times U_i)}{L_S}, \]  

where \(U\) is the highest velocity maintained for the whole interval (cm s\(^{-1}\)), \(T\) is the time elapsed at fatigue velocity (s), \(T_i\) is the prescribed interval time between each speed increment (300 s or 900 s), \(U_i\) is the velocity increment (5.5 cm s\(^{-1}\)), and \(L_S\) is the fish standard length (cm). No correction for blocking effect was applied since the total cross-sectional area of the fish did not exceed 5% of the swimming chamber (Bell & Terhune, 1970).

EVALUATION OF SWIMMING CAPACITY

Following the assessment of measurement repeatability, the fish used for the validation tests were directly transferred into salt water (SW; salinity 20, 6.8 ± 0.3°C). Salinity was adjusted by mixing St. Lawrence estuarine water (salinity 31–32) with dechlorinated FW before it entered rearing tanks. After a 48 h acclimation period, fish subgroups were submitted to the \(U_{\text{crit}}\) test as described above (Table I). As one fish reached exhaustion, it was rapidly removed from the flume and anaesthetized in 3-aminobenzoic acid ethyl ester (MS-222; 0.12 g l\(^{-1}\)) until opercular movements ceased (~ 1.5 to 2 min) for blood and tissue samplings. Control fish were submitted to the same \(U_{\text{crit}}\) procedure described above in FW, but only one group of 10 fish per cross-type swam together for these trials (Table I). Fish were not fed for 48 h before their transfer to the swimming chamber. To avoid circadian bias in hormonal
measurements, SW and FW $U_{\text{crit}}$ 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 ($\alpha=0.05$) 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* ($L_\varphi L_\sigma$) than in resident fish ($R_\varphi R_\sigma$) (Table III). $CF$ of $R_\varphi L_\sigma$ hybrids was similar to the paternal line ($L_\varphi L_\sigma$), while that of $L_\varphi R_\sigma$ 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 $R_\varphi L_\sigma$ having a higher $I_C$ than $L_\varphi R_\sigma$ 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 $U_{\text{crit}}$ values were similar in pure crosses of the anadromous and resident strains, swimming performance was 18% lower in $L_\varphi R_\sigma$ compared to the reciprocal $R_\varphi L_\sigma$. Also, swimming performance was significantly higher in FW ($3.50 \pm 0.13 \text{ } Ls \text{ } s^{-1}$) compared to SW ($3.00 \pm 0.07 \text{ } Ls \text{ } s^{-1}$) (Fig. 2).

STRESS AND OSMOTIC RESPONSE
Cortisol concentration was similar among all groups that underwent the swim challenge both in FW and SW (Table IV), with an overall mean of $6.25 \pm 0.60 \mu g \, dl^{-1}$. Even though significant treatment effects were found (Table IV), multiple comparison tests did not indicate differences in plasma glucose between the different cross-types and salinity groups. The overall mean plasma glucose was $0.90 \pm 0.04 \, mg \, ml^{-1}$.

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

Blood haematocrit varied according to cross-type (Table IV) and was positively correlated to body mass. Blood haematocrit was 12% lower in L♂L♀ fish (the smallest cross-type) than in the other cross-types (Fig. 4a). Blood haemoglobin varied according to both cross-type and salinity (significant interaction between factors), and a significant positive body mass covariance effect was noted (Table IV). In SW, blood haemoglobin concentration was highest in L♂R♀ hybrids while no difference could
be seen among cross-types in fish that swam in FW (Fig. 4b). The resulting MCHC differed among cross-types but not salinities: there was no significant covariate effect for body mass (Table IV). MCHC was 16% lower in R♀L♂ than in L♀R♂ hybrids, and MCHC levels in hybrids were similar to their respective maternal line (Fig. 4c).

ENERGY RESERVES

A significant interaction between cross-type and salinity was observed for muscle glycogen content with no body mass covariance effect (Table IV). After fish were challenged in FW, muscle glycogen content was 64.4% lower in anadromous and R♀L♂ hybrids than in R♂R♂ fish (Fig. 5a). The muscle glycogen content in the other hybrid was intermediate to those of the parental lines. Following exhaustion in SW, muscle glycogen content was similar among cross-types (Fig. 5). Within each cross-type, muscle glycogen content was similar whether swimming challenges were performed in FW or SW. A significant interaction between cross-type and salinity was also observed for liver glycogen content along with a significant positive body mass covariance effect (Table IV). Exhaustion in SW or FW only had a distinct effect in L♀R♂ hybrids, for which liver glycogen was 60% lower after the SW challenge compared to the concentration in fish exercised in FW (Fig. 5b). In FW-exhausted fish, liver glycogen was ~ 60% lower in Laval fish than in the three other cross-types, while liver glycogen concentration in SW was 56% lower in L♀L♂ and L♀R♂ than in the two other cross-types.

METABOLIC RESPONSE

There was a significant interaction between cross-type and salinity for white muscle CS capacity (Table IV). In FW, CS capacity was 27% higher in the Rupert fish (R♂R♂) than in the other cross-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 $L_x L_y$ 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 $L_x L_y$ fish had 66% less muscle lactate compared to the $R_y R_y$ and $L_x R_y$ cross-types while the concentration in $R_x L_y$ 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 $L_x R_y$ hybrids was 3.7 times lower than in the $R_x R_y$ cross-type (Fig. 7b), but there was no difference among cross-types following exhaustion in SW. Within cross-types, only $L_x R_y$ 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 $R_y L_y$ 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 $R_y L_y$ 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 $L_x L_y$ fish than in
hybrids. Globally, heart pyruvate concentration was 34.6% lower after the SW swimming challenge than after the FW challenge (Fig. 7e). This resulted in the highest heart lactate/pyruvate ratio for R♂L♂ hybrids challenged in SW (twice as high as the overall mean ratios of all other challenged fish) (Fig. 7f).

DISCUSSION

The main objective of this study was to test for the occurrence of functional divergence in the factors affecting swimming performance (estimated by \( U_{crit} \)) between pure strains and reciprocal hybrids issued from two wild populations of \( S. fontinalis \) having different migratory lifestyles (Laval strain: anadromous; Rupert strain: freshwater resident). Pure cross types had similar swimming performance in FW, and swimming performance was reduced by 14% following abrupt transfer to SW in both anadromous and resident fish. However, the pure cross types reached similar swimming speeds using different physiological strategies, suggesting different genetically-based physiological solutions to the same functional challenge. While no evidence was found for extreme non-additive genetic effects (i.e. heterosis or outbreeding depression) in hybrids, significant differences between the two reciprocal hybrids (L♂R♂ vs R♂L♂) were noted, with lower performance in L♂R♂.

PURE STRAINS

Fishes swimming performance is controlled by a number of physiological, morphological, and behavioural traits, all of which interact and involve potential trade-offs (Walker, 2010; Dalziel et al., 2011; Marras et al., 2013). Considering the principle of many-to-one mapping, many different combinations of traits can generate equivalent performance and multiple underlying factors can affect a 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
performance similar to that of anadromous fish due to their greater anaerobic capacities, but also because of higher energy reserves. The glycogen levels in epaxial muscle and liver following FW exercise were more than twice as high in resident than in anadromous fish. The exception was the epaxial muscle of resident fish tested in SW, which may indicate greater energetic demand following this trial. Thus the resident population compensated for its lower natural swimming ability (compared to the anadromous population) by having a higher metabolic capacity.

For species moving between FW and SW, a large osmoregulatory capacity is an additional and critically important determinant for maintaining swimming performance (Brauner et al., 1992; Nelson et al., 1996; McKenzie et al., 2001b; Chatelier et al., 2005). Regardless of FW rearing conditions, cross-type differences in the stress response to SW transfer were expected and a lower SW swimming performance in resident fish. Following the SW challenge, resident fish had plasma osmolality similar to anadromous fish combined with a gill Na\(^+\)-K\(^+\)-ATPase activity that was 4.4 times higher. However, no differences in other stress indicators were observed whether fish were exercised in FW or in SW. One may ask why experimental animals were reared in FW. In captivity, rearing 0+ and 1+ animals for prolonged periods in SW greatly increased events of opportunistic myxobacteria infections, suggesting impaired homeostasis, which is why young stages are routinely maintained in FW (C. Audet, unpublished data). Otherwise, 2+ and older anadromous Laval fish (including breeders) are reared at a salinity of 20 between the beginning of June and late September, mimicking the migration pattern of this wild anadromous fish population (Curry et al. 2010).

Previous studies comparing the performance of anadromous and resident populations in different fishes species showed that anadromous fishes possessed significantly greater swimming capacities than those from resident populations (O. kysutch: Taylor & Foote, 1991; S. fontinalis, Salmo trutta, S. salar: Peake et al., 1997; G. aculeatus: Dalziel et al., 2011; Kitano et al., 2012). It has been hypothesized that
their exposure to fast-water habitats, which are more energetically costly, allowed the anadromous 
fishes to evolve more efficient swimming abilities than resident populations (O. kysutch Taylor & 
Foote, 1991; S. fontinalis, S. trutta, S. salar, Peake et al., 1997; S. fontinalis: Morinville & Rasmussen, 
2003; 2008). In the present study, even though the swimming performance was similar between 
anadromous and freshwater resident fish, the results indicate a higher contribution of non-aerobic 
pathways in resident fish which suggests that they may be less adapted to sustained swimming.

REINORD HYBRIDS

Swimming performance and its underlying traits were different between the reciprocal hybrids. 
Compared to R_L hybrids, L_R hybrids had a 20% lower swimming speed, which was associated 
with a 24% smaller cardio-somatic index, a 21% higher MCHC, and a 19% higher haemoglobin 
concentration when swimming in SW as well as a larger metabolic (1.9 times higher muscle lactate 
accumulation) and energetic (44% less liver glycogen in SW) response. L_R hybrids thus expended 
greater effort and still had a lower performance than the reciprocal hybrid. Therefore, this performance 
depends on cross direction (parental line used as dam or sire). Such cross-direction phenomena have 
also been reported in M. salmoides (Cooke et al., 2001) and Chinook salmon Oncorhynchus 
tshawytscha (Walbaum 1792) (Falica & Higgs, 2012), but hybrids can often be similar in their 
swimming performance (Hawkins & Quinn, 1996; Dalziel et al., 2011). The reciprocal effect may be 
explained by various factors such as maternal or paternal effects, or genetic linkage between sex genes 
and performance genes. Swimming performance may be influenced by maternal effects, which are 
often involved in cross direction. However, these effects generally occur during early life development 
(due to egg size or yolk quality) with a decrease over time, and thus should probably be negligible in 
the present study since fish were tested at age 1+ (Taylor & Foote, 1991; Heath et al., 1999; Perry et 
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
resulted from acclimation. More recent studies have suggested that genetic adaptation could occur very quickly, e.g., within a small number of generations (Reznick et al., 1997; Pearse et al., 2009; Ellner et al., 2011; Westley et al., 2013). Since the separation of the S. fontinalis populations used in this study occurred around 10 000 years ago (Castric & Bernatchez, 2003), it seems that such a time frame would have been sufficient for the different populations to evolve distinct genetically based physiological adaptations to cope with their respective environments.

Differences between the two populations could be the results of local adaptation to different migratory lifestyles. Since swimming performance integrates the actions of a large number of organs and supporting functions, the investigation of the variability in swimming capacity within and among populations can be considered as a relevant means to reveal elements of local adaptation (Cooke et al., 2001; Odell et al., 2003; Pon et al., 2007). Although this needs to be more rigorously investigated, ecological differences in the populations’ migratory conditions (i.e., differences in fluctuations of temperature, velocity, and salinity experienced by the anadromous and the resident populations in their respective environments) could have influenced the physiological processes involved in swimming performance. Since the resident population likely faces strong currents during spring, swimming ability probably remained a key determinant of fitness for freshwater residency. However, it should be noted that the crosses in this study were only between the Rupert and the Laval strains. It is possible that crosses involving different anadromous and resident S. fontinalis populations could lead to results different from what was found here. Thus the possibility exists that the differences observed between the Rupert and Laval strains might not be linked to their migratory behaviour but to other forces shaping local adaptation. The Rupert and Laval fish used for this study were F3 fish, and domestication effects may already be present (Sauvage et al., 2010). However, other studies done on the same families have shown that they are still very different in terms of reproductive period, stress response (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).
Acknowledgements

The authors would like to thank I. Redjah, D. Lavallée, and N. Morin for their help with sampling and technical assistance. This work was supported by a strategic research grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada to Bernatchez, Audet, and collaborators (322102-05), by Ressources Aquatiques Québec (RAQ), a research network funded by the Fonds de Recherche du Québec – Nature et Technologies, by the Society for Experimental Biology (SEB), and by The Company of Biologists (COB).

References


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.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>$L_{\varphi}L_{\varphi}$</th>
<th>$L_{\varphi}R_{\varphi}$</th>
<th>$R_{\varphi}L_{\varphi}$</th>
<th>$R_{\varphi}R_{\varphi}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeatability test 1 (FW)</td>
<td>$n = 2 \times 15$</td>
<td>$n = 2 \times 15$</td>
<td>$n = 2 \times 15$</td>
<td>$n = 2 \times 15$</td>
</tr>
<tr>
<td>Repeatability test 2 (FW)</td>
<td>$n = 2 \times 15$</td>
<td>$n = 2 \times 15$</td>
<td>$n = 2 \times 15$</td>
<td>$n = 2 \times 15$</td>
</tr>
<tr>
<td>Repeatability test 3 (FW)</td>
<td>$n = 2 \times 15$</td>
<td>$n = 2 \times 15$</td>
<td>$n = 2 \times 15$</td>
<td>$n = 2 \times 15$</td>
</tr>
<tr>
<td>$U_{crit}$ (SW)</td>
<td>$n = 2 \times 15$</td>
<td>$n = 2 \times 15$</td>
<td>$n = 2 \times 15$</td>
<td>$n = 2 \times 15$</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$U_{crit}$ (FW)</td>
<td>$n = 1 \times 10$</td>
<td>$n = 1 \times 10$</td>
<td>$n = 1 \times 10$</td>
<td>$n = 1 \times 10$</td>
</tr>
</tbody>
</table>
Table II: Repeatability of critical swimming speed ($U_{\text{crit}}$, $L_S$ s$^{-1}$) in the two purebred strains of *S. fontinalis* ($L^\varphi L^\varphi$ and $R^\varphi R^\varphi$) and their reciprocal hybrids ($L^\varphi R^\varphi$ and $R^\varphi L^\varphi$). The repeatability tests were done in fresh water. Mean ± SE. $U_{\text{crit}}$ among trials were not statistically different.

<table>
<thead>
<tr>
<th></th>
<th>$L^\varphi L^\varphi$</th>
<th>$L^\varphi R^\varphi$</th>
<th>$R^\varphi L^\varphi$</th>
<th>$R^\varphi R^\varphi$</th>
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<tbody>
<tr>
<td>$n$</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>$U_{\text{crit}}$ 1</td>
<td>2.85 ± 0.21</td>
<td>2.83 ± 0.20</td>
<td>3.08 ± 0.13</td>
<td>2.24 ± 0.11</td>
</tr>
<tr>
<td>$U_{\text{crit}}$ 2</td>
<td>2.59 ± 0.18</td>
<td>2.65 ± 0.17</td>
<td>3.00 ± 0.17</td>
<td>1.90 ± 0.11</td>
</tr>
<tr>
<td>$U_{\text{crit}}$ 3</td>
<td>2.22 ± 0.15</td>
<td>2.47 ± 0.10</td>
<td>3.13 ± 0.18</td>
<td>2.44 ± 0.11</td>
</tr>
</tbody>
</table>

$n = \text{the number of individuals per swim test}$
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 (\(\alpha = 0.05\)).

<table>
<thead>
<tr>
<th></th>
<th>L(_L)</th>
<th>L(_R)</th>
<th>R(_L)</th>
<th>R(_R)</th>
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<tr>
<td>(n)</td>
<td>38</td>
<td>40</td>
<td>40</td>
<td>38</td>
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<tr>
<td>(L_S)(cm)</td>
<td>11.08 ± 0.16(^a)</td>
<td>13.29 ± 0.34(^c)</td>
<td>12.00 ± 0.24(^b)</td>
<td>11.94 ± 0.21(^b)</td>
</tr>
<tr>
<td>(M_B)(g)</td>
<td>11.11 ± 0.61(^a)</td>
<td>21.98 ± 1.98(^c)</td>
<td>13.63 ± 0.91(^a)</td>
<td>17.30 ± 0.95(^b)</td>
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<tr>
<td>(CF)(g cm(^{-3}))</td>
<td>0.79 ± 0.02(^a)</td>
<td>0.86 ± 0.02(^b)</td>
<td>0.76 ± 0.03(^a)</td>
<td>0.98 ± 0.02(^c)</td>
</tr>
<tr>
<td>(I_C)(%)</td>
<td>0.15 ± 0.01(^ab)</td>
<td>0.14 ± 0.01(^a)</td>
<td>0.18 ± 0.01(^b)</td>
<td>0.16 ± 0.01(^ab)</td>
</tr>
</tbody>
</table>

\(n = \) the number of individuals
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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cross-type effect</th>
<th>Salinity effect</th>
<th>Cross-type ×Salinity</th>
<th>Body mass covariable</th>
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<tr>
<td></td>
<td>$F$</td>
<td>df</td>
<td>$P$</td>
<td>$F$</td>
</tr>
<tr>
<td>$U_{crit}$</td>
<td>2.86</td>
<td>3</td>
<td>0.04</td>
<td>11.85</td>
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<tr>
<td>Cortisol</td>
<td>1.19</td>
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<tr>
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<tr>
<td>Muscle water</td>
<td>2.12</td>
<td>3</td>
<td>0.1</td>
<td>33.9</td>
</tr>
<tr>
<td>Osmolarity</td>
<td>5.1</td>
<td>3</td>
<td>&lt;0.01</td>
<td>96.35</td>
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<tr>
<td>Gill Na⁺K⁺ATPase</td>
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<td>3</td>
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<td>0.91</td>
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<td>4.6</td>
<td>3</td>
<td>&lt;0.01</td>
<td>3.51</td>
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<tr>
<td>Haemoglobin</td>
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<td>3</td>
<td>0.49</td>
<td>2.51</td>
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<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>n</td>
<td>SEM</td>
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<tr>
<td>------------------</td>
<td>------</td>
<td>-----</td>
<td>---</td>
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</tr>
<tr>
<td>MCHC</td>
<td>5.11</td>
<td>3 &lt;0.01</td>
<td>6.04</td>
<td>1</td>
</tr>
<tr>
<td>Muscle glycogen</td>
<td>5.47</td>
<td>3 &lt;0.01</td>
<td>5.23</td>
<td>1</td>
</tr>
<tr>
<td>Liver glycogen</td>
<td>14.27</td>
<td>3 &lt;0.01</td>
<td>9.94</td>
<td>1</td>
</tr>
<tr>
<td>CS</td>
<td>11.11</td>
<td>3 &lt;0.01</td>
<td>10.14</td>
<td>1</td>
</tr>
<tr>
<td>LDH</td>
<td>16.44</td>
<td>3 &lt;0.01</td>
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<tr>
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<td>14.5</td>
<td>3 &lt;0.01</td>
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<td>1</td>
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<tr>
<td>Muscle pyruvate</td>
<td>0.51</td>
<td>3 0.67</td>
<td>2.52</td>
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<tr>
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<td>3 0.08</td>
<td>4.88</td>
<td>1</td>
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<tr>
<td>Heart lactate</td>
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<td>3 0.87</td>
<td>2.04</td>
<td>1</td>
</tr>
<tr>
<td>Heart pyruvate</td>
<td>6.07</td>
<td>3 &lt;0.01</td>
<td>40.33</td>
<td>1</td>
</tr>
<tr>
<td>Heart ratio L/P</td>
<td>6.06</td>
<td>3 &lt;0.01</td>
<td>55.49</td>
<td>1</td>
</tr>
</tbody>
</table>
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 (α = 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 (α = 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 (α = 0.05).
Figure 5: (a) Muscle and (b) liver glycogen concentration 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 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 indicate respectively 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.
Figure 2

$U_{\text{crit}} (L_S \text{ s}^{-1})$

<table>
<thead>
<tr>
<th></th>
<th>LL</th>
<th>LR</th>
<th>RL</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td></td>
<td></td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>ab</td>
<td></td>
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<tr>
<td>ab</td>
<td></td>
<td></td>
<td>ab</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3

Na\textsuperscript{+}-K\textsuperscript{+} ATP-ase capacity
($\mu$M phosphate mg protein$^{-1}$ h$^{-1}$)

Osmolality (mosmol kg$^{-1}$)

(a)