Do Local Adaptation and the Reproductive Tactic of Atlantic Salmon (*Salmo salar* L.) Affect Offspring Metabolic Capacities?

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ABSTRACT

Atlantic salmon (*Salmo salar* L.) is an iteroparous, anadromous species that exhibits some of the greatest within-population variability in size and age at maturity of all vertebrates. In the conditional reproductive strategy of salmonids, the male reproductive tactic expressed is believed to depend on an individual male's status relative to others in the population and therefore depends on his capacity to attain a physiological threshold, the exact nature of which is unknown. Although the threshold is influenced by local biotic and abiotic conditions, it is likely to be under genetic control. Our study examined whether the early growth, muscle metabolic capacities, routine metabolic rate, and spontaneous swimming of salmon alevins reared in laboratory conditions varied with the population of origin, maternal investment, and the paternal reproductive tactic. Our experimental design allowed us to establish that neither the population of origin nor the paternal reproductive tactic influenced the physiological capacities of alevins. The strong influence of the mother on alevin metabolic capacities suggests that the bioenergetic differences in metabolic capacities, realized metabolic rates, and activity levels that could eventually dictate the reproductive tactic of male offspring may originate in maternal effects.

Introduction

Environmental heterogeneity influences all levels of biological organization from physiology through behavior to population dynamics. Two major mechanisms make it possible for a species to adjust to environmental variations: (1) phenotypic plasticity, where the characteristics of an individual change according to environmental conditions; and (2) evolutionary responses, whereby phenotypes change because of selection over successive generations (Stearns 1989; Hendry and Stearns 2004). Phenotypic plasticity includes direct responses to environmental conditions as well as transmissible epigenetic changes in gene expression. A "reaction norm" is the range of phenotypes produced by a given genotype exposed to varying environmental conditions (Schlichting and Pigliucci 1998; Pigliucci 2005). The shape of the reaction norms may be genetically variable. Thus, different populations faced with distinct levels of environmental variability may exhibit population-specific reaction norms (Piché et al. 2008).

In fish, reproductive strategies are extremely varied. Following strong inter- or intrasexual selection, fish may follow more than one developmental pathway to reproductive maturity (Gross 1996). Maynard Smith (1982) explained the coexistence of alternative phenotypes in the context of game theory, whereby the reproductive investment of an individual depends on that of others in the population. Alternative strategies of reproduction are genetic programs that dictate the allocation of reproductive and somatic effort by the alternative phenotypes. These strategies are thought to operate through physiological mechanisms detecting suitable indexes to set individual tactics (Gross 1996). Atlantic salmon are thought to follow a conditional strategy with partially heritable alternative tactics (Gross 1996; Gross and Repka 1998*a*, 1998*b*). Individual males "choose" a tactic based on their status (condition, size, etc.) and invest differently in growth and reproduction according to the tactic chosen. The fitness associated with each tactic is not equal, but the tactic "chosen" is thought to maximize the individual's fitness (Gross 1996).

The Atlantic salmon is an iteroparous, anadromous species that exhibits among the greatest within-population variability in size and age at maturity of all vertebrates (Fleming 1998; Garant et al. 2003). After a freshwater juvenile stage of 1–4 yr and a sea migration of 1–3 yr, large anadromous males return to their natal river to spawn (Gross 1985, 1991; Fleming et al. 1996; Garant et al. 2003). Some males become sexually mature before migrating to sea, at a younger age and at a size 5–10 times smaller than their anadromous counterparts (Jones and Orton 1940; Garant et al. 2002; Aubin-Horth and Dodson 2004). Lower rates of predation and mortality in freshwater may bring the lifetime reproductive success of mature male parr to similar levels as those of anadromous males (Hutchings and Myers 1994). The link between growth rate, size, and the selection of male reproductive tactic may vary with local conditions. Thus, in certain areas, virtually all male parr mature and remain in freshwater (Aubin-Horth et al. 2005). A combination of additive genetic effects (parental life history) and

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habitat quality shapes juvenile growth rate, which together with a mobile, genetically influenced threshold for tactic choice seems to be the main determinant of male reproductive tactics in salmon (Garant et al. 2003; Aubin-Horth and Dodson 2004).

The genetic influence on the "choice" of reproductive tactic is not direct, because male offspring of precocious male parr do not all follow in their father's path. As the threshold used to "choose" a reproductive tactic seems to be based on size or energetic status, genetic control of these parameters would influence which tactic is chosen. Sexual maturation of parr is encouraged by fast growth in freshwater (Whalen and Parrish 1999; Aubin-Horth and Dodson 2004; Aubin-Horth et al. 2005). A field study using otolithometry showed that parr fathered by mature male parr grow faster during early development than those fathered by anadromous males (Garant et al. 2002). On the other hand, studies with artificially bred progeny that used size or mass as a measure of growth showed diverse results, ranging from greater growth for the progeny of the most successful male parr (Garant et al. 2002) to greater growth for the offspring of anadromous males (Morasse et al. 2008). The multiple determinants of growth rate, both endogenous and exogenous, are likely to account for these divergent results.

Growth can be accelerated by increasing rates of feeding or by decreasing metabolic rates. The ^{137}Cs mass balance method shows that in the wild, mature Atlantic salmon parr have higher feeding and growth rates but lower metabolic efficiencies than nonmaturing parr (Tucker and Rasmussen 1999). On the other hand, Atlantic salmon smolts that migrate early have higher standard metabolic rates compared with those that migrate later (Metcalfe et al. 1992; Metcalfe et al. 1995). Migrating brook trout *Salvelinus fontinalis* show higher feeding rates but lower metabolic efficiencies than their resident counterparts (Morinville and Rasmussen 2003). Between hatching and first feeding, the energetic expenditures of alevins are a primary determinant of growth rates. Given the finite amount of vitellus, greater embryonic growth could be achieved by decreasing metabolic expenditures, including those required for maintenance and activity. Thus, to achieve faster growth, offspring of mature parr should have routine metabolic rates and spontaneous swimming activity during embryonic development that is lower than offspring of anadromous males.

By providing their offspring with substantial quantities of vitellus, salmonid females ensure that their offspring attain a considerable size before exogenous feeding. As large females typically have larger eggs, maternal size has a major influence on early larval growth (Thorpe 1987; Morasse et al. 2008). Population differences in the incidence of freshwater maturity (Aubin-Horth et al. 2006) could reflect a cascade of maternal, environmental, and genetic effects, combining large egg volumes, high food availability, and a genetic predisposition to fast growth. We reasoned that as the bulk of the fish is muscle, characteristics favoring growth should enhance the amount of muscle at a given age and enhance muscle metabolic capacities. Such influences of maternal and paternal characteristics on offspring growth would be particularly apparent while the offspring are entirely dependent on the vitellus and are growing in a common environment.

Our study examined whether early growth, muscle metabolic capacities, organismal metabolic capacities, routine metabolic rate, and spontaneous swimming of salmon alevins varied with the population of origin, maternal investment (egg volume), and paternal reproductive strategy. We compared offspring of anadromous females, anadromous males, and mature male parr from two recently (6 generations) diverging subpopulations of Atlantic salmon that reproduce in upstream and downstream areas of the Ste. Marguerite River, Canada. Offspring were reared under hatchery and laboratory conditions and characterized at 50% hatch and at 50% emergence. We measured rates of routine oxygen consumption and spontaneous swimming of alevins as well as parameters directly related to growth (mass, length, and protein content). We used enzymatic indicators to evaluate the metabolic capacity of the alevins, examining activities in the caudal and anterior sections as well as whole-body activities. We used the activity of creatine kinase (CK) as a proxy for muscular development. Aerobic capacity was revealed by cytochrome *C* oxidase (CCO) and citrate synthase (CS), glycolytic capacity by lactate dehydrogenase (LDH), and the capacity for ammonia metabolism by glutamate dehydrogenase (GDH).

Material and Methods

Habitat Characteristics, Breeding and Holding Conditions, and Sampling Times

The Ste. Marguerite River (48°20′N, 70°00′W) lies 250 km northeast of Quebec City, Canada and is divided into two branches. Access of salmon to the northeast branch is limited to the first 35 km because of waterfalls and was restricted to the lower 6.5 km until a fish ladder was installed at the first waterfall in 1981. Partial reproductive isolation between the upstream and downstream populations, as indicated by differentiation at microsatellite loci, was apparent in 1997 (Garant et al. 2002). The upstream site has a higher proportion of mature male parr and a smaller size threshold for maturation than the downstream site (Aubin-Horth et al. 2006).

During the summer of 2004, toward the end of the migratory period and when most of the adults had reached their spawning sites, anadromous males and females as well as mature parr were collected at the fish ladder and well downstream of the fish ladder. Fish collected from the fish ladder were regarded as representative of the upstream population. We collected four anadromous males, two anadromous females, and four mature male parr as broodstock for each population. These fish were kept in tanks at a fish hatchery in Tadoussac (15 km from the Ste. Marguerite River). Unfortunately, only two anadromous males from the upstream population survived, reducing the number of families we were able to produce (Table 1). The lengths and masses of the parental fish were measured before obtaining the eggs and sperm in November 2004. Crosses within each population were performed to yield full-sib, half-sib, and unrelated offspring with a projected hatching date in

Table 1: Time (d) to 50% hatching and 50% emergence in alevins from the different families produced from the upstream and downstream populations of Atlantic salmon

Population, Mother,[a] Father	Time to 50% Hatching	Time to 50% Emergence
Upstream:		
UF1:		
Anadromous 1	148	192
Anadromous 2	148	195
Mature parr 1	149	191
Mature parr 2	149	195
Mature parr 4	149	195
UF2:		
Anadromous 1	142	188
Mature parr 1	142	190
Mature parr 2	142	190
Mature parr 3	141	190
Mature parr 4	142	191
Downstream:		
DF1:		
Anadromous 1	150	199
Anadromous 3	149	196
Anadromous 4	149	196
Mature parr 1	150	199
Mature parr 2	154	198
Mature parr 3	149	195
Mature parr 4	153	198
DF2:		
Anadromous 1	146	194
Anadromous 2	145	194
Anadromous 3	145	195
Anadromous 4	146	196
Mature parr 1	147	195
Mature parr 2	147	195
Mature parr 3	148	196
Mature parr 4	148	196

[a] UF = upstream female, DF = downstream female.

late March 2005. The fertilized eggs were placed in drawers in the stream-fed incubator and maintained in darkness, except that after 24 h, nonfertilized eggs were removed. At the eyed stage (mid-March 2005), embryos were transferred to the Laboratoire Régional des Sciences Aquatiques (LARSA) at Université Laval to complete development. Egg volumes for each family were estimated from photos ($n = 280$) taken at the eyed stage (D. Pàez, personal communication).

At LARSA, the fertilized eggs were placed in a long, rectangular raceway. The embryos from each family were placed in compartments separated from the other families by netting. Well-aerated water at 4°C circulated through the entire raceway, assuring equivalence of the physiochemical conditions. Embryos were held in complete darkness except when incubation compartments were cleaned under a red light. During reproduction, larval rearing, characterization, and sampling, we followed procedures established by the Canadian Council for Animal Care and our local animal care committee (Comité de Protection des Animaux de l'Université Laval).

We characterized 15–20 alevins from each family at hatching and emergence. Each family was sampled as it reached 50% hatching and 50% emergence (as assessed by visual examination of the alevins). We took the absence of an external yolk sac as indicative of emergence. Alevins were sampled before exogenous feeding. Time to these developmental milestones differed little among the families (Table 1). At the times of sampling, none of the families showed any evidence of bimodality in the size-frequency distribution of the alevins.

Measurements of Spontaneous Swimming Activity

At hatch, the alevins moved little. To assess their activity, we filmed each family for 15 min while it was kept in its section of the raceway, placing the camera (Canon Optura Xi, mini

DV) directly above the tank. At emergence, spontaneous movements of six individuals per family were observed in separate petri dishes (9 cm) filled with water from the incubator and kept at 4°C. After a 5-min habituation period, fish were filmed for 15 min, following Morasse et al. (2008). During analysis of these films, movements were counted only if they were independent of previous movements (at least 5 s between movements). By using the mean number of movements, mean duration of displacements, and the total duration of the films, we calculated the percentage of time fish spent moving as an index of their spontaneous swimming activity.

Determination of Routine Metabolic Rate

At hatching and emergence, we measured the routine metabolic rates of 15–20 alevins per family. Individual plastic respirometry chambers (35 mL) were equipped with a false bottom of netting to separate the fish from the stirring bar. To maintain a constant temperature, these chambers were placed in a water bath in which water from the holding tanks circulated continuously. Magnetic stirrers were used to circulate the water in the respirometry chambers. Fish were habituated to the open respirometry chambers for 1 h before the chambers were closed with the polarographic electrodes (Clark-type electrode, Yellow Springs Instrument) and oxygen uptake was measured for 1 h.

The oxymeter (OM200 Oxygen Analyzer, Cameron Instruments) was calibrated to 100% saturation by using the aerated water from the incubator and adding sodium hydrosulfite to obtain 0% oxygen saturation. The calibration was performed at the start and the end of each day. To control for microbial O_2 uptake and O_2 uptake by the electrodes, we determined the oxygen consumption of empty chambers for 1 h before and after each sampling period. The water temperature in the respirometry chambers was 4°C. The atmospheric pressures for the sampling days were obtained from the Service Météorologique du Québec.

Sampling of Alevins and Preparation for Enzymatic Assays

At the end of oxygen uptake measurements, alevins were killed by a flick of a finger on the head. No bleeding resulted from this treatment. Fish were measured and weighed after being patted with paper towels to remove excess water. At hatch, fish were weighed with and without the yolk. Immediately after weighing, the alevins were frozen at −80°C until assayed. We calculated the Fulton's condition factor (mass [g] × total length $[\text{cm}]^{-3} \times 100$).

Frozen samples were randomly chosen for enzymatic analysis to avoid measuring all the individuals in one family at the same time. Throughout preparations for enzymatic assays, alevins and samples were preserved on ice. Alevins were dissected into two sections (anterior and caudal) at the level of the dorsal fin insertion. The caudal section allowed us to estimate muscle activities, whereas the anterior section reflected the activities of the major organs and the digestive system. Both sections were immediately homogenized in 10 volumes of KH_2PO_4/K_2HPO_4 (100 mM), Triton X-100 (0.1% v/v), and EDTA (5 mM), pH = 7.0. The pH of all solutions was adjusted at room temperature (20°C). After homogenization, 50 μL of the homogenate was kept at −20°C for determination of protein concentration. Protein concentration was measured using bicinchoninic acid (Smith et al. 1985) after solubilizing protein in the homogenates with acetic acid, urea, and Triton X-100 according to Somero and Childress (1990). The remainder of the homogenates was centrifuged 10 min (500 *g*, 5°C; Micromax), and the supernatants were used for enzymatic tests.

Enzyme Activity Assays

Enzymes were assayed using a microplate UV/VIS spectrophotometer (SpectraMax 190, Molecular Device) coupled to Softmax Pro software for data recovery (ver. 4.6, Molecular Device). Assays were performed at 20°C. All assays were linear with respect to homogenate volume. Estimates of activity were based on the initial 4 min during which optical density change was linear with time. CS activity was measured at 412 nm to detect the transfer of sulfhydryl groups to 5,5′dithiobis-2-nitrobenzoic acid (DNTB; micromolar extinction coefficient = 13.6). CCO activity was measured at 550 nm to follow the oxidation of reduced cytochrome *C* (micromolar extinction coefficient = 19.1). GDH, LDH, and CK activities were measured by following NADH and NADP at 340 nm (micromolar extinction coefficient = 6.22).

The assay conditions were based on Guderley et al. (2001) and Kuo et al. (1994): CCO: 100 mM KH_2PO_4/K_2HPO_4, pH = 7.5, 0.075 mM cytochrome *C* reduced with sodium hydrosulfite (excess sodium hydrosulfite was removed by bubbling the cytochrome *C* solution with air); CS: 100 mM Tris-HCl, 0.1 mM acetyl-CoA, 0.1 mM DNTB, 0.5 mM oxaloacetate (omitted for control), pH = 8.0; GDH: 50 mM imidazole-HCl, 250 mM ammonium acetate, 0.1 mM EDTA, 0.1 mM NADH, 1 mM ADP, 14 mM α-ketoglutarate (omitted for control), pH = 7.4; CK: 100 mM Tris-HCl, 3.3 mM $MgCl_2$, 0.3 mM NADP, 3.3 mM glucose, 0.75 mM ADP, 5 mM AMP, excess levels of glucose-6-phosphate dehydrogenase and hexokinase, 36 mM creatine-phosphate (omitted for control), pH = 7.5; LDH: 50 mM imidazole-HCl, 0.16 mM NADH, 4 mM pyruvate (omitted for control), pH = 7.5. All enzymes were measured in triplicate and in both sections of the alevins. Enzymes activities were expressed in international units (U; the amount of enzyme required to produce 1 μmol of product per min at 20°C). To estimate the total enzyme activity per alevin, we added the total units obtained for each section and calculated the specific activity by dividing by the alevin mass. Biochemical supplies were purchased from Sigma Chemicals, Roche Diagnostics, and Fisher Scientific.

Statistical Analysis

For statistical analysis, the normality of the residuals was tested with the Shapiro-Wilk test and the homogeneity of variances was tested with the Levene test. A mixed model containing

both fixed and random effects was used to evaluate the influence of the different parameters in our experimental design. The design used randomized blocks with fixed treatment effects and random block effects (SAS system for mixed models, 2002). For each sampling period, the fixed effects in the model were male reproductive phenotype (anadromous or mature parr), population of origin of the parents, mother's mass, egg volume, and alevin mass (when it is required). We also included maternal identity, paternal identity, and their interaction (e.g., family) as random effects. As the identity of the parents was seldom significantly linked with alevin characteristics, we simply report significant effects in the text and have not included them in the tables of statistical results (Tables 2–4). The significance of the effect of family identity (a random effect in the model) was determined using the likelihood-ratio χ^2 test. All models initially included a range of fixed and random effects. When factors such as alevin mass, maternal mass, or egg volume were not significant, they were removed from the model to establish whether the model was improved by their omission. The models reported in Table 2 showed no significant effect of alevin mass. The level of significance was $\alpha = 0.05$. Computations were performed with SAS 9.0 statistical software.

Results

Anatomical Parameters

Neither the population of origin, the paternal reproductive tactic (PRT), nor the interaction between population and PRT affected offspring mass, percent of vitellus, or condition factor (Table 2; Fig. 1*A*–1*C*). Mass of alevins at hatch (without vitellus; Fig. 1*A*) varied considerably among families (Table 2) and was negatively influenced by the mass of the mother ($r^2 = 0.12$; Table 2). Of all the factors we examined, only family identity influenced alevin mass at emergence (Table 2). At hatching, alevin condition factor (K) decreased with increasing maternal mass ($r^2 = 0.06$) and egg volume ($r^2 = 0.06$). At emergence, the mass of the mother retained a significant influence ($r^2 = 0.04$) and had a positive influence on condition, whereas the influence of egg volume disappeared. Family identity significantly affected condition factor only at emergence. The percent vitellus was positively correlated with the mother's mass ($r^2 = 0.10$; Table 2), and it varied with family identity. Similarly, neither protein content at hatch nor at emergence was affected by the population of origin, PRT, or their interaction (Table 3; Fig. 1*F*). Egg volume and alevin mass were positively linked with the total protein content of the alevins at hatching ($r^2 = 0.32$ and $r^2 = 0.02$, respectively); at emergence, total protein content was correlated only with alevin mass ($r^2 = 0.09$; Table 3).

Metabolic Rate

The metabolic rates were expressed in μmol O_2 h^{-1} g^{-1} and thus represent mass-specific metabolic rates and not total metabolic rates (Fig. 1*D*). We chose to present this parameter because total metabolic rate would have reflected both metabolic rate and offspring size. Both at hatching and emergence, offspring metabolic rates were significantly related to the mother's mass and the egg volume (Table 2). Both factors were negatively linked with metabolic rate. Thus, as maternal mass and egg volume increased, the metabolic rate of the resulting offspring decreased. Alevin mass was not significantly linked with these metabolic rates. Family identity did not affect metabolic rate either at hatching or at emergence. Neither the population, the

Table 2: Results (P values) of mixed models for morphologic parameters and metabolic rate and activity of juveniles at hatching and emergence

	Random Effects	Fixed Effects				
Parameters (n)	Family	Population	PRT	Population × PRT	Maternal Mass	Egg Volume
At hatching:						
Mass (381)	<.0001***	.230 (1)	.076 (9)	.168 (9)	.010 (358)**	/
% Vitellus (422)	<.0001***	.289 (1)	.122 (9)	.212 (9)	.014 (396)*	.185 (396)
K (409)	.527	.298 (1)	.897 (9)	.438 (9)	<.0001 (383)***	<.0001 (383)***
MO_2 (150)	≥.99	NE (1)	.468 (9)	.673 (9)	.020*	.009**
Activity (150)	<.0001***	.254 (1)	.040 (9)*	.118 (9)	/	/
At emergence:						
Mass (144)	.026*	.522 (1)	.857 (9)	.931 (9)	.562 (120)	/
K (428)	<.001***	NE (1)	.974 (9)	.785 (9)	.003 (402)**	.614 (396)
MO_2 (150)	≥.99	NE	.673	.454	<.0001***	<.0001***
Activity (145)	≥.99	.509	.718	.638	/	/

Note. n = total number of individuals at hatching and at emergence; NE = nonestimable values in the model; / = a fixed effect not included in the final mixed model for a variable. Values of the fixed effects are accompanied by degrees of freedom in parentheses, where applicable. For the condition factor, K, values were log transformed before statistical tests were performed, but untransformed values are shown in Figure 2. Metabolic rate (MO_2) at hatching is expressed over mass without vitellus. PRT = paternal reproductive tactic.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 3: Results (P values) of mixed models for protein contents of juveniles at hatching and at emergence

	Random Effects	Fixed Effects				
Protein (n)	Family	Population	PRT	Population × PRT	Offspring Mass	Egg Volume
At hatching (211)	.107	.306 (1)	.770 (9)	.824 (9)	<.0001 (187)***	.042 (187)*
At emergence (143)	≥.99	.456 (1)	.167 (9)	.399 (9)	.0002 (118)**	.477 (118)

Note. n = total number of individuals at hatching and at emergence. Values of the fixed effects are accompanied by degrees of freedom in parentheses. PRT = paternal reproductive tactic.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

PRT, nor an interaction between the two influenced offspring metabolic rate at hatching or emergence. The marked differences in metabolic rate (Fig. 1*D*) are thus linked to the different mothers through their mass and the volume of the eggs they produced.

Rates of Spontaneous Activity

At hatching, family identity and PRT significantly affected the spontaneous activity of the alevins (Table 2; Fig. 1*E*). Offspring of precocious males were more active than those of anadromous males. At emergence, neither family identity nor any of the fixed effects (e.g., population, PRT, alevin mass, mother's mass, or egg volume) affected the spontaneous activity of the offspring (Table 2). However, the identity of the mother and that of the father significantly influenced the spontaneous activity of the offspring.

Enzyme Activity

Here, only the results of the total enzymatic activities are presented because the statistical analyzes of the enzymatic activities in the anterior and caudal sections gave the same conclusions as the analysis of total activities. Both at hatching and at emergence, the primary factor affecting whole-body enzyme activities (U g^{-1}) was the family; in other words, the interaction between paternal and maternal identity (Table 4; Fig. 2). This effect was highly significant, with P values below 0.0001 for all enzymes at hatching and $P < 0.0003$ for all enzymes except CS and GDH at emergence. The mother's mass had a significant effect on the activity of CS and LDH at hatching and on the activity of CCO, CS, and GDH at emergence. The influence of maternal mass on enzyme activities was negative, both at hatching and at emergence. Offspring mass influenced the activity of CK and LDH at hatch, whereas at emergence it influenced CCO, CS, and GDH activity. The influence of offspring mass was positive at hatching but became negative at emergence, except for with CCO, for which the relationship remained positive. As CK and LDH are primarily found in muscle, whole-organism activities will reflect the extent of muscle growth. When expressed against the levels of a mitochondrial enzyme that should be present in all tissues, the ratios CK/CS and LDH/CS become a proxy for the extent of muscle development. Again, we detected no statistical influence of the population or of the PRT, but there was marked variability between the families.

To examine whether the changes in enzyme activity reflected a qualitative change in the proteins produced in the different families, we analyzed the enzyme activities expressed relative to protein content. As proteins were measured after solubilization with urea and acetic acid (Somero and Childress 1990), the value includes both soluble and structural proteins. The statistical effect of family identity was markedly reduced when enzyme activities were expressed relative to the protein content and was only significant for two enzymes (GDH and CK) and only at hatch (Table 5). The effect of offspring mass was significant for all enzymes, but only at hatch. For enzyme activities expressed relative to 1 mg protein, the mass of the mother had no influence, and so this factor was removed from the mixed model.

Links between Parameters Measured at Hatching and Emergence

The mass and condition of offspring from a given family at hatching and emergence were not correlated. The average metabolic rates per family at hatching were not correlated with the rates measured at emergence. For the different enzymes measured, the average activities at hatching were not correlated with the activities measured at emergence except for CS, for which the activities measured at the two periods (U g^{-1} wet mass) were positively correlated. Thus, families that had high CS activities at hatching maintained high activities at emergence.

Correlations between Metabolic Rate, CCO, and CS Activities

Routine metabolic rate was positively correlated with CS activity (U g^{-1} wet mass) both at hatching ($P = 0.04$) and at emergence ($P = 0.003$). CCO activity was not linked with routine metabolic rates. At hatching, the activities of CS and CCO were positively correlated ($P < 0.0001$), but this relationship was not apparent at emergence. No other enzyme activities were correlated with metabolic rates.

Table 4: Results (P values) of mixed models for potential factors, both fixed and random, determining the whole-body specific enzyme activity (U g^{-1} wet mass) of juveniles at hatching and emergence

	Fixed Effects	Random Effects				
Enzyme (n)	Family	Population	PRT	Population × PRT	Offspring Mass	Maternal Mass
At hatching:						
CCO (345)	<.0001***	.631 (1)	.737 (9)	.420 (9)	.084 (321)	.457 (321)
CS (349)	<.0001***	.386 (1)	.294 (9)	.459 (9)	.794 (325)	.0002 (325)***
GDH (350)	<.0001***	.789 (1)	.077 (9)	.480 (9)	.178 (326)	.730 (326)
CK (344)	<.0001***	.948 (1)	.172 (9)	.607 (9)	.018 (320)*	.734 (320)
LDH (349)	<.0001***	.474 (1)	.040 (9)*	.304 (9)	.0001 (325)***	.004 (325)**
At emergence:						
CCO (144)	<.0001***	.451 (1)	.579 (9)	.437 (9)	.064 (119)	.074 (119)
CS (144)	.011*	.294 (1)	.021 (9)*	.038 (9)*	.0001 (119)***	<.0001 (119)***
GDH (144)	.032*	.615 (1)	.199 (9)	.645 (9)	.0001 (119)***	<.0001 (119)***
CK (144)	<.0001***	.366 (1)	.065 (9)	.142 (9)	.080 (119)	.248 (119)
LDH (144)	.0003***	.359 (1)	.575 (9)	.394 (9)	.456 (118)	.141 (118)

Note. n = total number of individuals analyzed. Values of the random effects are accompanied by degrees of freedom in parentheses. PRT = paternal reproductive tactic. CCO = cytochrome C oxidase; CS = citrate synthase; GDH = glutamate dehydrogenase; CK = creatine kinase; LDH = lactate dehydrogenase. As egg volume had no influence on these values, it was removed from the model.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Correlations between Swimming Activity and Other Parameters

The percentage of time spent moving at hatching was positively correlated with the activity of CCO (U g^{-1} wet mass; $r^2 = 0.108$; $P = 0.0002$), with LDH activity ($r^2 = 0.052$; $P = 0.011$), and with protein content ($r^2 = 0.067$; $P = 0.0026$). Not surprisingly, percentage of time spent moving decreased with increases in the % vitellus ($r^2 = 0.065$; $P = 0.0021$). At emergence, the percentage of time spent moving was positively correlated with routine metabolic rate ($r^2 = 0.077$; $P = 0.001$) but negatively correlated with CK activity ($r^2 = 0.068$; $P = 0.002$).

Discussion

Salmonids can specialize for habitats they have not previously inhabited in as little as 60–100 yr, or approximately 13–30 generations (Hendry et al. 2000). In the Ste. Marguerite River system, the installation of a fish ladder 23 yr before our study allowed Atlantic salmon to exploit an upstream habitat that was previously inaccessible. Although the upstream and downstream populations of Atlantic salmon in the Ste. Marguerite River have diverged genetically (Garant et al. 2002) and although the upstream populations show a greater incidence of maturity of male parr (Aubin-Horth et al. 2006), none of the physiological parameters we measured were affected by the population from which the parents originated. These parameters included offspring mass at hatch and emergence, offspring condition, spontaneous swimming activity, routine metabolic rates, protein content, and glycolytic and mitochondrial enzyme activities. Enzyme activities were measured in the anterior and caudal sections we sampled and were calculated for the entire alevin. None of these ways of examining enzyme activities revealed any population differences. PRT had little effect on the physiological characteristics of the offspring sampled at hatch and emergence; it affected only the spontaneous swimming activity of the alevins at hatch. This spontaneous swimming was, in turn, positively linked with size, but only at hatch. Spontaneous swimming is positively related to alevin mass and protein content in comparisons of normal and dechorionated trout embryos (Ninness et al. 2006). The lack of an effect of PRT on swimming activity at emergence suggests that this effect is short lived. If stronger effects of PRT or population of origin appear in field-sampled juveniles, they must reflect an interaction between the genotype and the environment.

Knowledge of characteristics that influence the reproductive tactic chosen by male parr is central in understanding the variability of population size structure in rivers inhabited by Atlantic salmon. Current theory suggests that the "choice" of reproductive tactic is based on the status of an individual male parr relative to a threshold whose exact level varies with the population under study (Aubin-Horth and Dodson 2004). The choice of reproductive tactic by a male parr has been suggested to be influenced by the reproductive tactic of his father (Glebe and Saunders 1986), and such an influence seemed apparent early during ontogeny (Garant et al. 2002). Early rapid growth seems to predispose male parr to early maturity, whereas slow growing male parr become anadromous (Rowe and Thorpe 1990; Aubin-Horth and Dodson 2004). Our study of the influence of PRT on the physiological characteristics of offspring reared under controlled laboratory conditions allowed us to focus on genetic differences. Our 25 independently reared families revealed no influence of PRT on metabolic rate, protein content, alevin mass, or enzyme activities. Even the activity of CK, our proxy for muscle growth, was not influenced by the

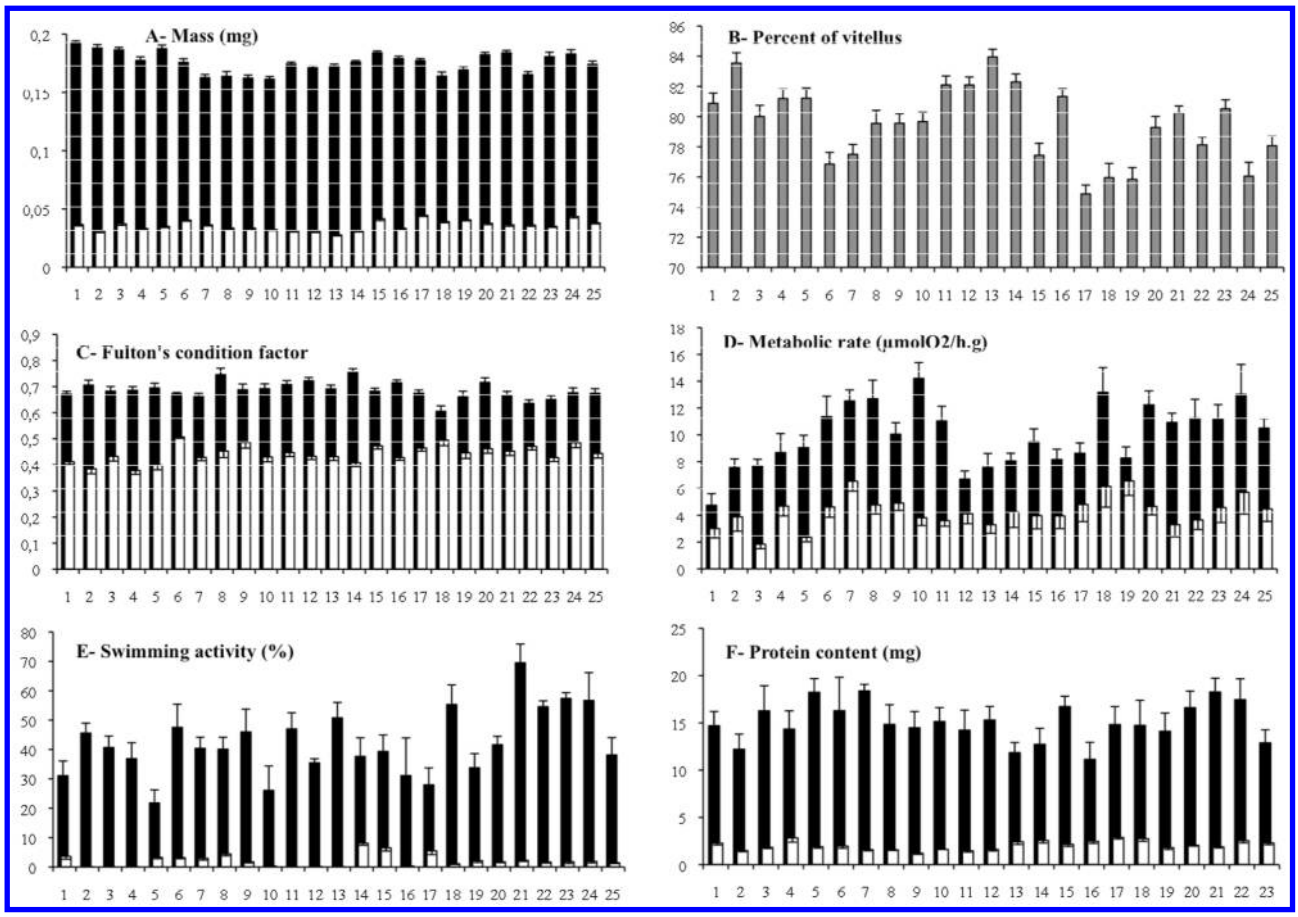

Figure 1. Anatomic parameters, metabolic rates, and swimming activity shown by family (numbers in *X*-axis). Open bars represent parameters at hatch, and filled bars represent parameters at emergence. Mass (mg) of alevins (*A*), percent of total mass as vitellus at hatch (*B*), Fulton's condition factor (K = [body mass × length^{-3}] × 100; *C*), metabolic rate of offspring (μmol O_2 h^{-1} g^{-1}; *D*), voluntary swimming activity (% time spent swimming; *E*), and protein content (mg) per offspring (*F*). Values are means ± SE. Mass and protein content at hatch do not include vitellus. The families numbered 1–10 represent the upstream population; the remaining numbers represent families in the downstream population.

PRT. Previous studies obtained different results. In 2002, Garant et al. (2002) concluded that offspring of mature male parr grew faster than those of anadromous males, whereas in 2008, Morasse et al. (2008) found that offspring of anadromous males had better muscle development and swam more than offspring of mature male parr. These studies used different designs: Garant et al. (2002) was a field-based study in which fertilized eggs were sampled after natural mating, whereas Morasse et al. (2008) followed development in the laboratory but used fewer families and allowed sperm competition among the mature male parr during the in vitro fertilizations. Our characterization of specific in vitro crosses, in the absence of sperm competition or natural mate choice, strongly suggests that the physiological characteristics of alevins did not reflect the PRT. Thus, if the "choice" of reproductive tactic is set by size or metabolic characteristics early in ontogeny, our results suggest that paternal tactic does not influence the "decision."

Despite the lack of an influence of PRT or population of origin on the physiological characteristics, the interfamily variability of these characteristics was pronounced, suggesting that some families would have much higher rates of early maturity than would others. This interfamily variability was pronounced for most characteristics and could be explained by a paternal effect in only one case (e.g., spontaneous activity of alevins at emergence). Maternal effects mediated by the mass of the mother or the volume of the eggs influenced numerous physiological characteristics at hatch and maintained considerable influence at emergence. Maternal investment in yolk production is of critical importance for the survival of the developing young (Jobling 1994). Accordingly, we found alevin mass, condition, protein content, metabolic rate, and LDH activity at hatching to be affected by maternal mass. At emergence, condition, metabolic rate, CS, and GDH also showed an influence of maternal mass. Effects of egg volume accompanied those of maternal mass. Links between egg volume and GDH activity suggest that the dynamics of nitrogen metabolism and the capacity for production, storage, and elimination of urea may differ among families. Except in the case of the largest female, we observed a positive relation between the mass of the female and the volume of her eggs. Female salmon size is known to

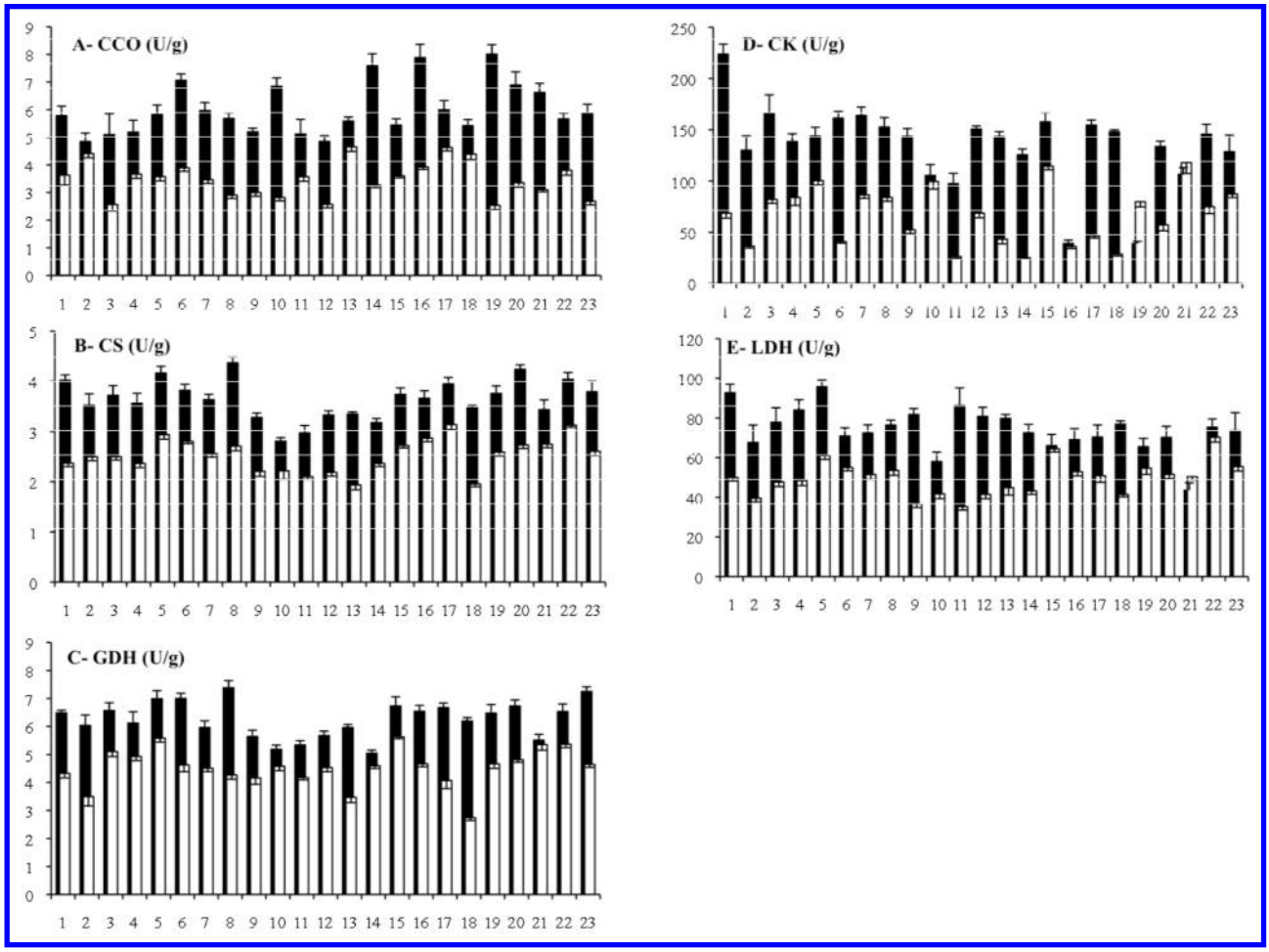

Figure 2. Whole-body specific enzyme activities at hatching (*open bars*) and at emergence (*filled bars*) shown by family (numbers in *X*-axis). Values are means ± SE, $n = 15$–20 individuals per family at hatching, and $n = 6$ per family at emergence. Enzyme activities of cytochrome *C* oxidase (*A*), citrate synthase (*B*), glutamate dehydrogenase (*C*), creatine kinase (*D*), and lactate dehydrogenase (*E*) are shown as U g^{-1} wet mass and represent the combined activities for the caudal and anterior sections. The families numbered 1–10 represent the upstream population; the remaining numbers represent families in the downstream population.

influence breeding success in many ways (Thorpe et al. 1983). Egg quality, as evaluated by its energy content, is positively dependent on female size (Heinimaa and Heinimaa 2004). In natural salmon populations, egg number and size may be dependent on female size (Thorpe et al. 1983; but see Heinimaa and Heinimaa 2004). Juvenile size, in turn, is positively correlated with egg size (Einum and Fleming 1999, 2000*a*, 2000*b*).

Metabolic capacities of offspring as estimated by maximal enzyme activities (U g^{-1} alevin mass) differed considerably between families at hatch and emergence. However, expressing enzyme activities relative to offspring protein content (U mg^{-1} protein) markedly attenuated these interfamily differences. When enzyme activities were expressed relative to protein content, only GDH and CK showed significant interfamily differences, and only at hatch. Thus, the composition of alevin proteins varied less among families than did protein content. Protein content can thus be seen to determine most enzyme activities (U g^{-1}). Both at hatch and emergence, alevin mass was the principal determinant of protein content. The parameters that determine alevin mass would thereby determine alevin metabolic capacities. Pàez et al. (2010) show that maternal effects have a significant influence on yolk-sac size and on alevin growth between hatch and emergence in the same Atlantic salmon populations we examined. Our results also show numerous maternal effects on offspring size, condition, and protein content and, by extension, on offspring metabolic capacities.

By measuring traits at different levels of organization, we were able to assess whether variation at the metabolic level was reflected in organismal performance. Such links included a positive correlation between routine metabolic rate and CS activity at hatching and emergence. This correlation could reflect the costs of maintaining a large mitochondrial population, but the lack of correlation between CCO activity and metabolic rate casts some doubt on this interpretation. At hatch, CCO and LDH activities were correlated with an index of swimming activity. Protein content at hatching was also correlated with swimming activity. At emergence, routine metabolic rate was

Table 5: Results (*P* values) of mixed models for potential factors, both fixed and random, determining the protein-specific whole-body enzyme activity (U mg^{-1} protein) of alevins sampled at hatching and emergence

	Fixed Effects	Random Effects				
Enzyme (*n*)	Family	Population	PRT	Population × PRT	Offspring Mass	Egg Volume
At hatching:						
CCO (345)	.388	.404 (1)	.459 (9)	.053 (9)	<.0001 (167)***	.001 (167)**
CS (349)	.052	.279 (1)	.809 (9)	.808 (9)	<.0001 (170)***	.011 (170)*
GDH (350)	<.0001***	.524 (1)	.903 (9)	.393 (9)	<.0001 (169)***	.015 (169)*
CK (344)	<.0001***	.552 (1)	.189 (9)	.247 (9)	<.0001 (170)***	.147 (170)
LDH (349)	.100	.332 (1)	.329 (9)	.230 (9)	<.0001 (169)***	.009 (169)**
At emergence:						
CCO (144)	.149	.773 (1)	.143 (9)	.233 (9)	.418 (119)	.581 (119)
CS (144)	>.99	.801 (1)	.588 (9)	.602 (9)	.333 (119)	.041 (119)*
GDH (144)	>.99	.947 (1)	.332 (9)	.330 (9)	.253 (119)	.081 (119)
CK (144)	.061	.601 (1)	.241 (9)	.373 (9)	.446 (119)	.689 (119)
LDH (144)	.905	.863 (1)	.177 (9)	.872 (9)	.975 (118)	.568 (118)

Note. *n* = total number of individuals. Values of the random effects are accompanied by degrees of freedom in parentheses. PRT = paternal reproductive tactic. CCO = cytochrome *C* oxidase; CS = citrate synthase; GDH = glutamate dehydrogenase; CK = creatine kinase; LDH = lactate dehydrogenase.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

positively correlated with percentage of time swimming. Because metabolic rate and swimming activity were measured on different individuals in different experimental setups, this positive correlation suggests that the metabolic cost of spontaneous swimming raises routine metabolic rates. There is thus some evidence that variation at the metabolic level translates into variation in organismal performance.

In summary, our experimental design allowed us to establish that neither the population of origin nor the PRT influenced the physiological capacities of alevins. The influence of PRT found in other studies (Garant et al. 2002; Morasse et al. 2008) seems to have been the result of genotype-environment interactions. The strong influence of the mother (direct, via egg volume or alevin protein content) on alevin metabolic capacities suggests that the bioenergetic differences in metabolic capacities, realized metabolic rates, and activity levels that could eventually dictate the reproductive tactic of male offspring may originate in maternal effects.

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