Paternal Reproductive Strategy Influences Metabolic Capacities and Muscle Development of Atlantic Salmon (*Salmo salar* L.) Embryos

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ABSTRACT

Male Atlantic salmon follow a conditional strategy, becoming either "combatants" that undertake a seaward migration and spend at least a year at sea or "sneakers" that remain in freshwater and mature as parr. A variety of physiological indices showed significant but small differences between the offspring of males that use these two reproductive tactics. Offspring fathered by anadromous male Atlantic salmon (*Salmo salar* L.) showed greater muscular development and muscle metabolic capacities but lower spontaneous movements than those fathered by mature male parr. At hatch and at maximum attainable wet weight (MAWW), offspring fathered by anadromous males had higher activities of mitochondrial (cytochrome C oxidase and citrate synthase) and glycolytic (lactate dehydrogenase [LDH]) enzymes than progeny of mature male parr. Enzymatic profiles of progeny of anadromous fathers also suggested greater nitrogen excretion capacity (glutamate dehydrogenase) and increased muscular development (creatine kinase and LDH) than in the progeny of mature parr. At MAWW, juveniles fathered by mature parr made considerably more spontaneous movements, presumably increasing their energy expenditures. For juveniles fathered by anadromous males, total cross-sectional areas of white and red muscle at hatch were higher due to the greater number of large-diameter fibers. We suggest that the slightly lower metabolic capacities and muscular development of alevins fathered by mature parr could reflect differences in energy partitioning during their dependence on vitellus. Greater spontaneous movements of offspring of mature male parr could favor feeding and growth after the resorption of the vitellus.

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Introduction

Many fish species show considerable plasticity in their reproductive tactics (Gross 1984, 1985; Sigurjónsdóttir and Gunnarsson 1989; Ryan et al. 1992; Fu et al. 2001). For example, in Atlantic salmon, *Salmo salar*, male reproductive phenotypes are extremely different and suggest marked energy trade-offs between investment in growth and sexual maturity (Glebe and Saunders 1986; Berglund 1992; Whalen and Parrish 1999; Garant et al. 2002; Aubin-Horth and Dodson 2004). Two tactics coexist in a given population and, most of the time, at the same spawning site (Taborsky 1998). The "fighting" tactic involves competition between males for access to nests and females. Sexual maturation of these anadromous males takes several years, of which two to three are spent in freshwater, followed by at least a year of accelerated growth in seawater. The surviving adults return to natal rivers to reproduce. On the other hand, many males mature earlier (1–3 yr) at a very small size and live entirely in freshwater. During spawning, these mature males "sneak" toward females to try to fertilize the eggs without being spotted by combatant males and can achieve considerable success (Flemming 1996). The frequency of early male maturity may vary from 9% to 50% for 1-yr-old salmon and between 13% and 100% for 2-yr-old salmon (collectively referred to as parr), with frequency increasing upstream (Aubin-Horth et al. 2006).

A genetically monomorphic conditional strategy is thought to govern the reproductive tactics of Atlantic salmon (Maynard Smith 1982; Gross 1991, 1996), providing an excellent example of phenotypic plasticity. The reproductive tactic is "chosen" according to the animal's status (e.g., size, condition factor, growth capacities, etc.) relative to others in the population. Theory assumes that this choice maximizes the individual's lifetime reproductive success that is, in turn, influenced by the tactics used by others in the population (negative frequency-dependent selection). The choice of tactic is dictated by an animal's capacity to attain a fixed physiological threshold. The position of the threshold is set by the biotic and abiotic environments and may be influenced by parental genotype (Gross and Repka 1998*a*, 1998*b*). In general, fast growth in freshwater encourages sexual maturation of parr (Berglund 1992; Whalen and Parrish 1999; Aubin-Horth and Dodson 2004). In the field, juveniles fathered by mature parr can grow faster (as estimated by otoliths) during early ontogenesis than those fathered by anadromous males (Garant et al. 2002). Faster growth of parr

destined to become sexually mature may be facilitated by higher food ingestion despite high metabolic costs imposed by greater swimming (Tucker and Rasmussen 1999).

In larval fish, swimming, growth, and all physiological processes are integrated into a tight energy budget (Wieser 1991, 1994, 1995), particularly while larvae depend on their vitellus. As a major proportion of somatic mass in fish is muscle (Johnston 2001), we reasoned that genetic factors affecting growth and bioenergetic strategies should be expressed within muscle and should influence muscle capacities. Thus, we examined whether offspring of Atlantic salmon fathered by mature parr demonstrate physiological characteristics linked with faster growth either during or after embryonic development. We reasoned that the influence of genetic determinants would be most apparent when the progeny are entirely dependent on the nutrients provided by the mother and are reared in a common environment. Thus, we compared offspring fathered by anadromous males with those fathered by mature parr at hatch and at maximum attainable wet weight (MAWW) during laboratory rearing. Although we did not aim to document maternal effects in this study, we nevertheless replicated the paternal comparisons by using two females to generate two maternally independent lineages, each composed of two paternal broodlines. We measured parameters directly related to growth (length, mass, and protein content) and used enzymatic indicators to evaluate the metabolic capacity of the alevins, focusing on aerobic capacity (as revealed by cytochrome C oxidase [CCO] and citrate synthase [CS]), glycolytic capacity (using lactate dehydrogenase [LDH] as an indicator), and nitrogen metabolism (as revealed by glutamate dehydrogenase [GDH]). We assessed the activities of creatine kinase (CK; together with those of LDH, CCO, and CS) and acetylcholinesterase (AChE) in the caudal section of the alevins as proxies for the development of muscle fibers and their innervation. The total caudal activity of CK and LDH gives insight into the amounts of red and white muscle. The mass-specific activities indicate the metabolic flux each enzyme can support (Patterson et al. 2004). Analysis of muscle cellularity (fiber number and area) compared muscle development in young from the two broodlines at hatch. Finally, we quantified spontaneous movements to verify whether offspring from these paternal broodlines differ in their activity.

Material and Methods

Breeding Conditions and Holding

In October 2003, adult salmon (females F01 and F02 and males MA01 and MA02 having migrated to sea [anadromous salmon], and mature parr MP01, MP02, MP03, and MP04) were captured on the northeastern branch of the Saint Marguerite River (48°20′N, 70°00′W), Québec, Canada (Table 1). Sperm of males employing the same reproductive tactic was mixed to form two batches. For each mature parr, sperm was completely withdrawn, whereas a portion (3 mL) of the sperm was taken from anadromous males. The two batches were used to fertilize 1,000 eggs from each female (total of 2,000 eggs, mixed beforehand).

Table 1: Weight of parental individuals and relative fertilization success of males

Parental Identity	Wet Weight (g)	Fertilized Eggs (%)
Mature parr 01 (MP01)	107.9	4.9
Mature parr 02 (MP02)	41.5	15.9
Mature parr 03 (MP03)	60.4	1.2
Mature parr 04 (MP04)	61.8	78.0
Anadromous male 01 (MA01)	1,950.0	56.7
Anadromous male 02 (MA02)	1,800.0	43.3
Female 01 (F01)	4,200.0	…
Female 02 (F02)	3,800.0	…

Adipose fins of parents were preserved in 95% ethanol for subsequent genetic analyses. Eggs were transported within 24 h to the Laboratoire Régional des Sciences Aquatiques, Laval University, Québec, Canada, and were incubated in drawers placed inside an environmental room at 4°C until hatch. Holding conditions were the same for both broodlines. During reproduction, larval rearing, characterization, and sampling, we followed the procedures established by the Canadian Council for Animal Care and our local animal care committee (Comité de Protection des Animaux de l'Université Laval).

Dates of hatch (50% hatch in drawers) and of MAWW were predicted using WinSIRP software (Jensen et al. 1992). Fifty percent hatch occurred at 118 d postfertilization (DPF) and MAWW at 196 DPF. Each brood line was sampled at the same date. Based on the measurement of morphological characters, such as total length, somite pairs, and caudal fin rays (Gorodilov 1996), the two broodlines did not differ in developmental rate, indicating that young-of-the-year salmon (hereafter referred to as alevins) fathered by anadromous males and by mature parr passed the major developmental milestones at the same time. At the times of sampling, neither broodline showed any evidence of bimodality in the size frequency distribution of the alevins.

Determination of Spontaneous Movements

At hatch, Atlantic salmon were practically motionless, so spontaneous movements were not measured. At MAWW, spontaneous movements of 50 alevins from each broodline were observed in 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, using a digital camera (PV-DV702-K, Panasonic). During analysis, individual movements were counted only if they were independent of previous movements (at least 5 s between two movements). The mean duration of the independent movements was calculated from 30 sequences

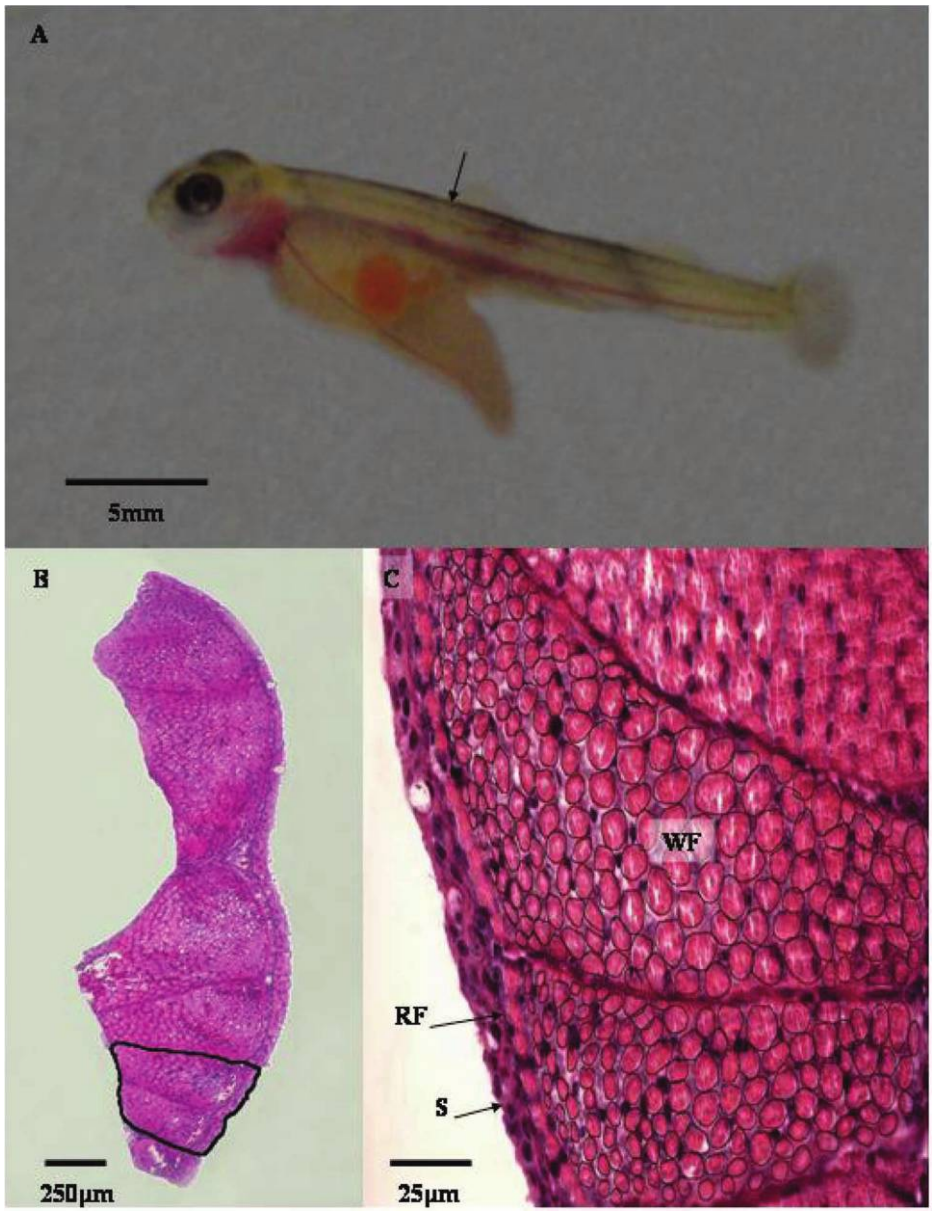

Figure 1. *A*, Body location of cross section used for muscle cellularity measurements (*arrow*) in Atlantic salmon alevins at hatch. *B*, *C*, Representative subsections sampled for the analysis of white fibers (*traced areas*). Single layer of red fibers (*RF*) under the skin (*S*) and white fibers located deeper (*WF*). Cross sections were stained with hematoxylin-eosin.

selected randomly (JMP IN, ver. 5.1, SAS Institute) for alevins from each broodline. This allowed estimation of percentage of time spent moving. The mean duration of a movement (4.4 ± 0.68 s) was similar for the two broodlines ($P = 0.31$).

Determination of Anatomical Parameters, Protein Content, and Enzyme Activity

For each sampling period, 50 fish from each broodline were euthanized by cervical dislocation and immediately frozen at −80°C until assays were performed. Before assays, fish were maintained on ice, lightly dried with tissue paper, and weighed before and after dissection of yolk. Length was measured using digital images of the fish taken while alive (ImageJ, ver. 1.33u, National Institutes of Health). Fish were dissected into caudal and rostral sections at the level of dorsal fin insertion. Both sections were immediately homogenized in 10 vols of KH_2PO_4/K_2PO_4 (100 mM), Triton X-100 (0.1% v/v), and EDTA (5 mM), pH 7.0. Homogenization was performed using a 2-mL ground-glass grinder. A 50-µL aliquot of the homogenate was frozen at −20°C for protein determination. Homogenates were centrifuged for 10 min at 500 *g* (Micromax, IEC), and the super-

Table 2: Weight, length, and protein content of offspring fathered by anadromous males and by mature parr and for progeny of each female

	Female Progeny		Anadromous Males Progeny	Mature Parr Progeny	τ_i	δ_k	$\tau\delta_{ik}$
	FA01	FA02					
Wet weight at hatch including yolk (mg)	126 ± 1	100 ± 1	108 ± 1	110 ± 1	F=370.83, P <.0001	F = .0056, P =.94	F = .73, P =.39
Wet weight at hatch excluding yolk (mg)	33.9 ± .7	30.0 ± .5	31.8 ± .6	30.9 ± .6	F = 22.22, P <.0001	F = 2.15, P =.15	F = .095, P =.76
Wet weight at MAWW (mg)	155 ± 2	133 ± 2	142 ± 2	146 ± 2	F = 45.73, P <.0001	F = .79, P =.38	F = .15, P =.70
Length at hatch (mm)	17.1 ± .4	16.7 ± .3	17.1 ± .4	16.7 ± .3	F = .54, P =.46	F = .38, P =.54	F = .91, P =.34
Length at MAWW (mm)	28.6 ± .3	27.4 ± .3	27.7 ± .3	28.3 ± .3	F = 7.50, P =.0076	F = 1.67, P =.20	F = .18, P =.67
Protein content at hatch excluding yolk (mg)	2.7 ± .1	2.4 ± .1	2.5 ± .1	2.5 ± .1	F = 7.94, P =.0061	F = .078, P =.78	F = .049, P =.83
Protein content at MAWW (mg)	18.3 ± .4	14.1 ± .4	16.1 ± .4	16.4 ± .4	F = 51.63, P <.0001	F = .025, P =.87	F = 2.43, P =.12

Note. τ_i = maternal identity; δ_k = paternal reproductive tactic. At hatch, n = 83; at maximum attainable wet weight (MAWW), n = 87. Values are mean ± SE.

natant was used for assays. The pellets were stored at −80°C for later genetic analyses.

Enzymatic assays were carried out in a temperature-controlled chamber at 20°C using a microplate spectrophotometer (SpectraMax 190, Molecular Devices; Softmax Pro ver. 4.6, Molecular Devices). Preliminary studies comparing activity at 5° and 25°C indicated that all enzymes maintained their activity at 25°C. Determination of GDH and LDH followed nicotinamide adenine dinucleotide (NADH) oxidation, while that of CK followed the production of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm (micromolar extinction coefficient = 6.22). CS and acetyl cholinesterase (AChE) activities were followed at 412 nm to detect transfer of sulfhydryl and thiocholine groups, respectively, to 5,5′dithiobis-2-nitrobenzoic acid (DTNB; micromolar extinction coefficient = 13.6). CCO activity was measured following oxidation of reduced cytochrome C at 550 nm (micromolar extinction coefficient = 19.11). The pH of all solutions was adjusted at 20°C and enzyme activity was determined from a linear portion of the curve that lasted at least 4 min. Enzyme extraction and assay conditions were based on work by Guderley et al. (2001), Lassiter et al. (2003), and Kuo et al. (1994). Maximum activities were obtained under the following conditions: (1) CS: 100 mM Tris-HCl, 0.1 mM DTNB, 0.1 mM acetyl CoA, 0.5 mM oxaloacetate (omitted for control), pH 8.0; (2) CCO: 75 mM KH_2PO_4/K_2PO_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); (3) CK: 100 mM Tris-HCl, 3.3 mM $MgCl_2$, 0.3 mM NADP, 3.3 mM glucose, 0.75 mM ADP, 5mM AMP, excess levels of glucose-6-phosphate dehydrogenase and hexokinase, 36 mM creatine phosphate (omitted for control), pH 7.5; (4) AChE: 100 mM KH_2PO_4/K_2PO_4, 0.1 mM DTNB, 2 mM acetylthiocholine (omitted for control), pH 8.0; (5) 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; and (6) LDH: 50 mM imidazole-HCl, 0.16 mM NADH, 4 mM pyruvate (omitted for control), pH 7.5.

All enzymes were measured in the caudal section, which is largely muscle and excludes the major organs. CCO, GDH, and CS activities were also measured in the rostral section to assess the organismal capacity for oxygen uptake and nitrogen metabolism. All assays were performed in duplicate. Enzyme activities were expressed in international units (1 IU is the amount of enzyme producing 1 μmol of product per min at 20°C). Protein determination used the bicinchoninic acid method (Smith et al. 1985).

Evaluation of Muscle Development

Muscle cellularity was studied using a modification of the protocol of Johnston and McLay (1997). Alevins were killed by cervical dislocation and fixed in Bouin's fluid. Following identification of the parents as described below, five alevins per family (eight families) were chosen for histological study. These samples were dehydrated in an ethanol gradient and impregnated with liquid paraffin. Cross sections (7 mm thick) were made, stained with hematoxylin and eosin, and mounted on slides. Cellularity was analyzed at the dorsal fin insertion (Fig. 1). Viewed with a binocular microscope, the dorsal fin was easily visible on the slides. The first slide showing the initial rays was selected for further analysis. Digital images of cross sections were taken using a microscope (DMRX, Leica) con-

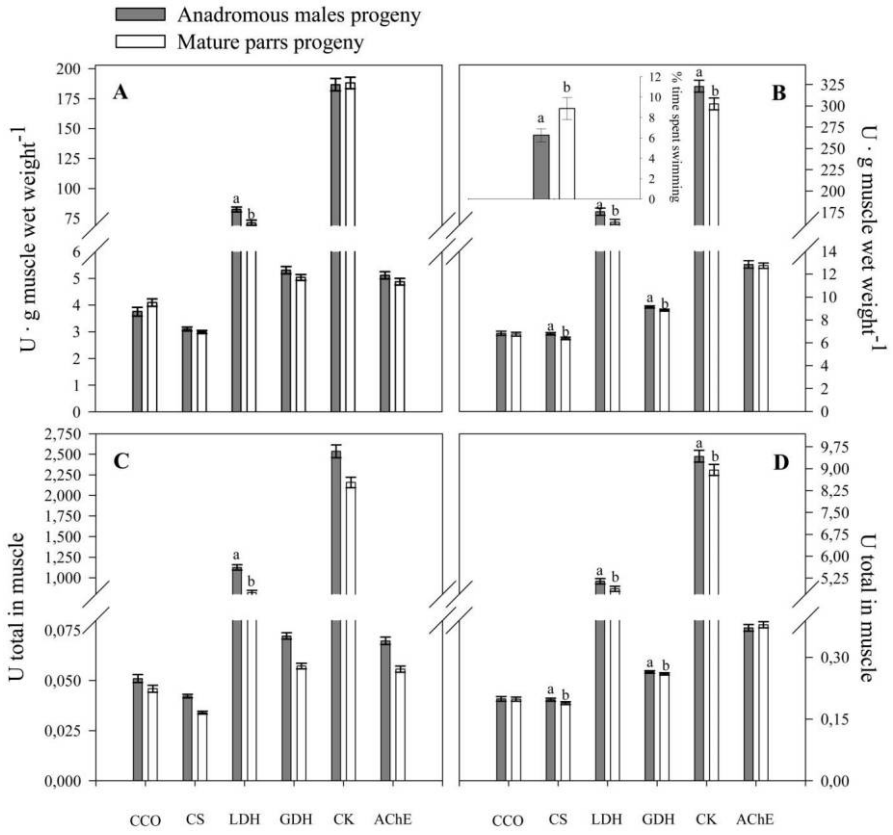

Figure 2. Effect of paternal reproductive tactics on spontaneous movements (*C*, *inset*) and on enzymatic profiles of offspring (caudal section). *A*, *B*, At hatch, $n = 83$; *C*, *D*, at maximum attainable wet weight, $n = 87$. Values are mean ± SE and are expressed in international units (μmol product min^{-1}). Significant differences are indicated by the columns for a given enzyme having different letters ($P < 0.05$).

nected to a camera (DC-300, Leica) and Image Manager Software (ver. 1.20, Leica). Two subsections were selected for analysis of white fiber number and area (Fig. 1). Johnston and McLay (1997) demonstrated that these sections show patterns that are representative of the whole cross-sectional area of white muscle in Atlantic salmon at hatch. The contour of each fiber located in these subsections was traced (magnification × 400). All red fibers in half of a cross section were analyzed in the same sample. Red fibers were clearly recognizable because of their size, position, and orientation in the muscle layer under the skin. Tracing was done with ImageJ, directly on the images.

The parameters measured within the cross sections were (1) total cross-sectional area of white muscle, (2) total cross-sectional area of red muscle, (3) cross-sectional area of white fibers in the representative subsections sampled, (4) cross-sectional area of red fibers, (5) total number of white fibers per cross section (deduced from the total area of white muscle and area of white fibers), and (6) total number of red fibers per cross section. Following Johnston and McLay (1997), we assumed bilateral symmetry for final calculation of these parameters.

Genetic Analysis

Microsatellite polymorphism was analyzed to determine parental identity of alevins and reproductive success of males a posteriori. Parental DNA extraction was performed using methods described by Sambrook and Russel (2001). Alevin DNA, either from fins preserved in 95% ethanol or from frozen homogenates, was extracted using extraction microplates from Qiagen and a vacuum pump (BAC_{96} Miniprep Kit, Millipore). We used three microsatellite loci developed for *Salmo salar* and known to be highly polymorphic (Ssa171, Ssa197, O'Reilly et al. 1996; SSOSL85, Slettan et al. 1995). Each polymerase chain reaction (PCR) was carried out (modified from O'Reilly et al. 1996 and Slettan et al. 1995) in 15 μL and included 1 unit of Taq DNA polymerase, 200 mM Tris-HCl, pH = 8.8, 1% Triton X-100, 500 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 2 μL of previously extracted total DNA, and primer concentrations ranging from 0.04 to 0.28 μM (Ssa171: 0.28 μM, Ssa197: 0.15 μM, SSOLS85: 0.04 μM). One 5′ primer for each locus was labeled either green (VIC) for Ssa171 and SSOSL85 or blue (6-FAM) for Ssa197. Multiplexed PCR (Touchgene Gradient Thermal Cycler, Techne) was used for Ssa171 and Ssa197 and followed these steps: initial denaturing for 3 min at 95°C and then 35 repetitions of a three-part cycle: 30 s at 94°C, 30 s at 56°C, and 30 s at 72°C. Allelic size was automatically determined (3100 Genetic Analyzer, ABI) and analyzed with Genemapper software (ver. 3.7, ABI). Parental genotypes proved to be so

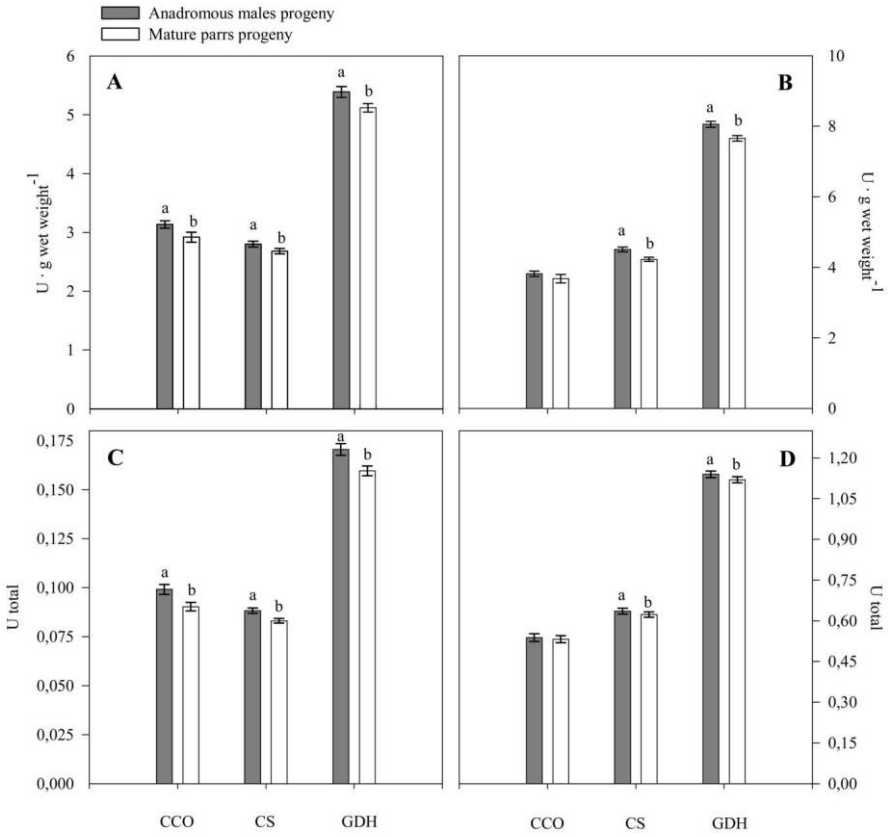

Figure 3. Effect of paternal reproductive tactics on enzymatic profiles of offspring (combined activities of caudal and rostral sections). *A*, *B*, At hatch, $n = 83$; *C*, *D*, at maximum attainable wet weight, $n = 87$. Values are mean ± SE and are expressed in international units (μmol product min^{-1}). Significant differences are indicated by the columns for a given enzyme having different letters ($P < 0.05$).

different that it was easy to determine parental identity of alevins. These assignments were nevertheless checked a posteriori using PAPA software (ver. 2.0; Duchesne et al. 2002).

Data Analysis

We used the MIXED procedure of the SAS software (ver. 9.0, SAS Institute), first testing the assumption that the random effect of "paternal identity" was equal to zero (Bernier-Cardou and Bigras 2001). After establishing this for each variable ($P < 0.15$), we tested the model:

$$Y_{ijkl} = \mu + (\tau_i + \delta_k + \tau\delta_{ik}) + \varepsilon_{l(ijk)}.$$

Data varied according to the general mean μ, the fixed effect of maternal identity τ_i, the fixed effect of paternal reproductive tactic δ_k, the interaction between maternal identity and paternal reproductive tactic $\tau\delta_{ik}$, and the residual error of the model $\varepsilon_{l(ijk)}$.

For analysis of mass-specific enzymatic activities in the caudal section, both caudal and body weight were covariables. Caudal weight was a covariable for total caudal enzymatic activity. Whole-body (both caudal and rostral sections) enzymatic activities were analyzed with body weight as a covariable. Analysis and incorporation of these covariables took both the allometric relations between mass and mass-specific enzymatic activity and the potential bias created by differential dissections of alevins into caudal and rostral sections into account.

Results

Fertilization Success and Anatomical Parameters

Genetic identification confirmed that fertilization success differed markedly between mature parr, MP04 being the father of 78.0% of offspring (Table 1). Anadromous male MA01 fathered more offspring than MA02 (57% vs. 43%), even though equal volumes of sperm were used from the two.

Paternal reproductive tactic had no significant effect on offspring wet weight, length, or protein content either at hatch or at MAWW (Table 2). However, for both sampling periods, these parameters were strongly influenced by female identity. The maternal effect was particularly marked for alevin wet weight (including yolk) at hatch. Female F01 provided more vitellus, facilitating offspring growth until MAWW. Not surprisingly, F01 was the larger female (Table 1).

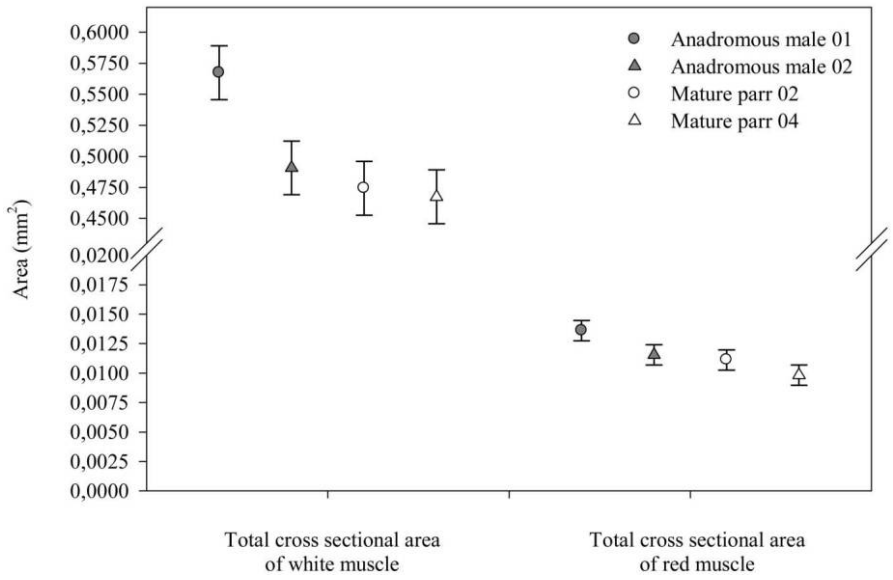

Figure 4. Total cross-sectional area of red and white muscles of offspring at hatch. Values are means ± SE, $n = 10$ for each group.

Enzymatic Activity in the Caudal Section at Hatch and MAWW

At hatch, the total and mass-specific activities of LDH in the caudal section were significantly higher in offspring fathered by anadromous males (Fig. 2*A*, 2*B*). This suggests a higher anaerobic capacity and more advanced muscular development in the anadromous lineage. This tendency occurred for almost all enzymes in the caudal section at hatch. As alevin size (heavily influenced by maternal identity) was included in all the ANCOVAs, maternal identity did not generally have a significant effect.

At MAWW, enzymatic differences between the broodlines became more generalized. Total and mass-specific activities of CS and LDH (Fig. 2*C*, 2*D*) suggested higher aerobic and anaerobic capacities as well as an increased quantity of muscle in offspring of anadromous males. CK activity, a direct indicator of muscle quantity, was higher in offspring from the anadromous lineage. As at hatch, GDH activity suggested an increased capacity to metabolize nitrogen in alevins fathered by anadromous males. AChE activity was not influenced by paternal tactic, either at hatch or at MAWW.

Whole-Body Enzymatic Activity at Hatch and MAWW

CCO, CS, and GDH were measured in the rostral and caudal sections. For all three, both the total and the mass-specific activities were significantly higher in the anadromous lineage at hatch. At MAWW, the activities of CS and GDH remained higher in the anadromous lineage (Fig. 3).

Spontaneous Movements at MAWW

At MAWW, progeny from the mature parr lineage showed more spontaneous movements (Fig. 2*C*, *inset*). The percentage of time spent moving during 15 min was significantly higher in offspring fathered by mature parr than in those from anadromous fathers. Short but very fast and abrupt movements characterized almost all observed activity.

Muscle Development and Cellularity at Hatch

The total cross-sectional area of white muscle was higher in offspring from anadromous males ($P = 0.016$; Fig. 4) fathered by MA01. The total cross-sectional area of red muscle was also higher in alevins fathered by anadromous males ($P = 0.020$). These results were reflected by a higher number of large fibers in offspring of anadromous males. The size distribution of white fibers was similar in the two broodlines (Fig. 5). However, when offspring of MP02 were removed from the analysis, the progeny of MP04 had a lower frequency of white fibers 10–12 μm in diameter than offspring of anadromous males. Large red fibers with diameters 11–14 μm were more frequent in offspring fathered by anadromous males than in other alevins ($P < 0.05$; Fig. 6), while small red fibers with diameters 3–4 μm were more frequent in the mature parr lineage ($P = 0.05$). Total areas of white and red muscle were moderately correlated with mean diameter of their constituent fibers in alevins fathered by anadromous males (Figs. 7, 8) but not at all in progeny of mature parr. Mean cross-sectional area of red fibers tended to be higher in alevins fathered by anadromous males (48.8 ± 2 μm^2 vs. 43.4 ± 2 μm^2; $P = 0.06$). For offspring of mature parr, increased total cross-sectional area of red muscle was positively correlated with an increase in red fiber number ($R^2 = 0.38$). However, neither

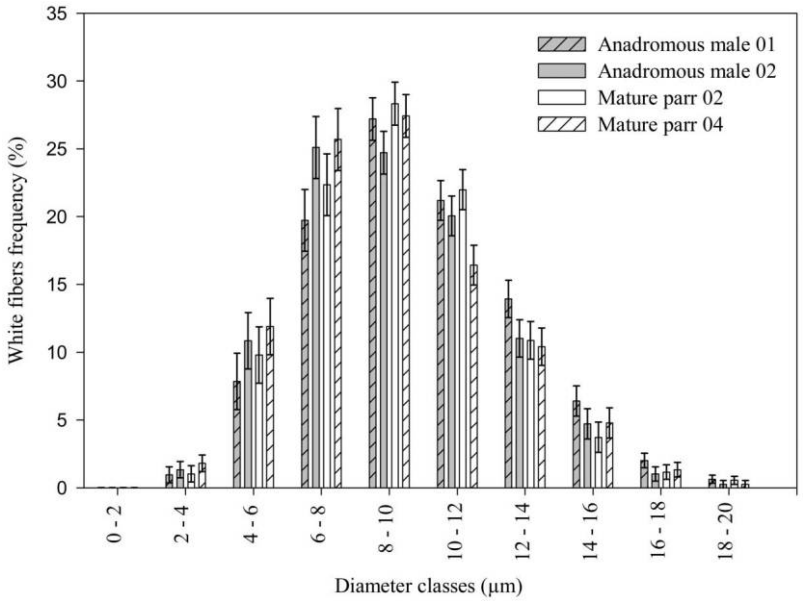

Figure 5. Frequency distribution of white fiber diameters of offspring at hatch. Values are means ± SE, $n = 10$ for each group.

broodline showed a significant correlation between total area of white muscle and white fiber number. The latter was estimated assuming that the sampled subsections were representative of the entire cross section (in terms of fiber number and size), whereas the number of red fibers came from a complete census. Finally, there was a tendency toward increased fiber density in alevins fathered by mature parr (8,643 ± 522 vs. 9,903 ± 522 fiber/mm^2; $P = 0.096$).

Discussion

The reproductive tactic of Atlantic salmon males influenced the metabolic capacities and muscle cellularity of their offspring both at hatch and at MAWW and also influenced their spontaneous movements at MAWW. Because individuals showing a faster growth rate are more likely to reach the decisional threshold in the first spring following hatch and to become

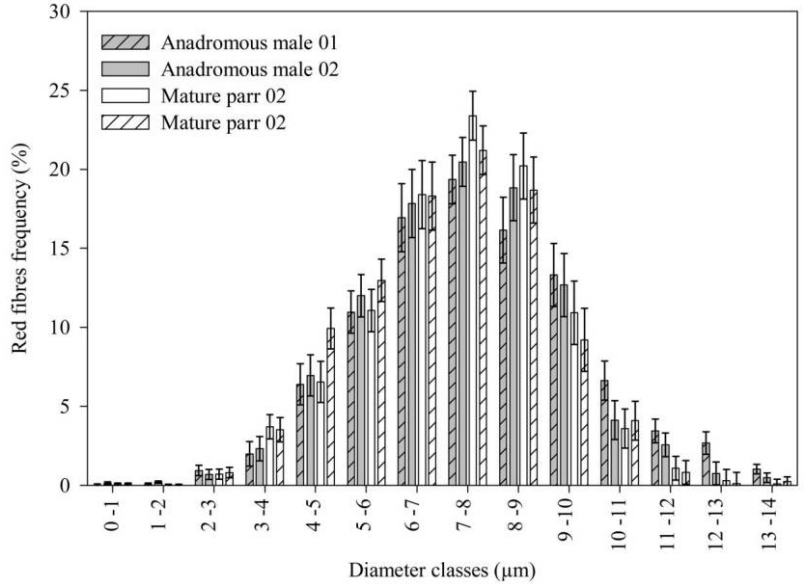

Figure 6. Frequency distribution of red fiber diameters of offspring at hatch. Values are means ± SE, $n = 10$ for each group.

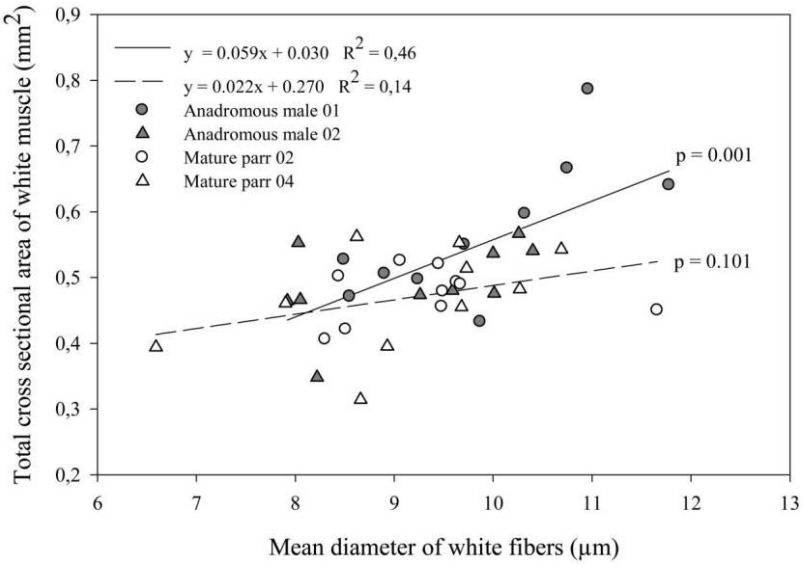

Figure 7. Total cross-sectional area of white muscle versus mean white fiber diameter of offspring at hatch.

sexually mature (Rowe and Thorpe 1990; Berglund 1992; Whalen and Parrish 1999; Aubin-Horth and Dodson 2004), our initial hypothesis was that offspring of mature male parr would grow faster and show the hallmarks of faster growth in their metabolic capacities and muscle cellularity. However, the weight, length, and protein contents of the progeny of anadromous males and mature male parr did not differ at hatch or at MAWW (Table 2). This held true even when considering only offspring fathered by the most successful parr (MP04). As in other studies, maternal identity had a major influence on all anatomical parameters (see Thorpe 1987). Offspring of the larger female (F01) had higher reserves at hatch and considerably greater size at MAWW. In contrast to our initial hypothesis, enzymatic activities and muscle cellularity were slightly, but significantly, greater in offspring of anadromous males than in those of mature male parr. The differences in enzyme activity were apparent at hatch and became more pronounced at MAWW.

Studies linking paternal reproductive tactic with growth of Atlantic salmon alevins have obtained a variety of patterns. Using otolithometry, Garant et al. (2002) found that alevins fathered by mature parr in their natural environment grew faster during yolk resorption than those fathered by anadromous males. In a second experiment with artificially bred progeny that used size as a measure of growth, Garant et al. (2002) found only an effect of paternal reproductive tactic on growth

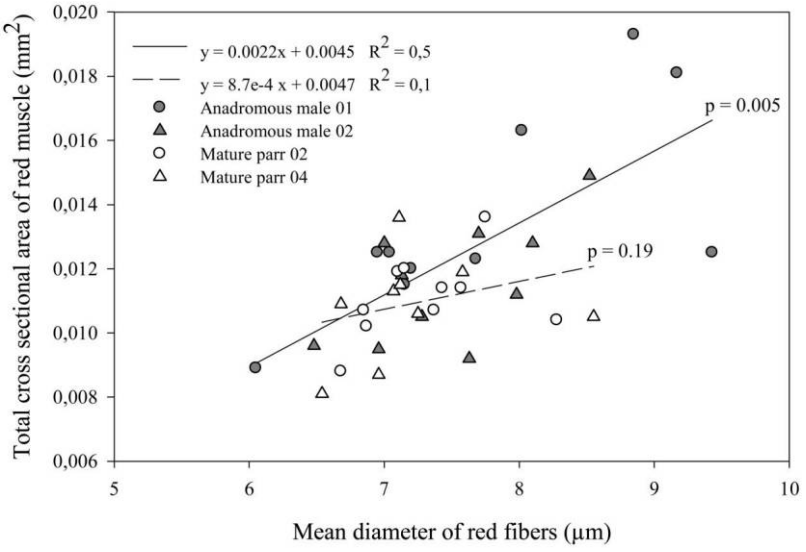

Figure 8. Total cross-sectional area of red muscle versus mean red fiber diameter of offspring at hatch.

between hatch and MAWW when focusing on the progeny of the most successful mature male parr. They ascribed these results to overrepresentation of potentially subordinate mature parr in the artificial crosses. Such a bias may have occurred in our results. However, as we allowed sperm competition between four mature parr, this effect should have been reduced. Alternatively, retrocalculation of growth in populations of individuals that have been subjected to size-selective mortality (Meekan et al. 1998; Good et al. 2001; Aubin-Horth et al. 2005) may provide a biased signal. Such biases could have led Garant et al. (2002) to overestimate the size of the alevins fathered by mature parr in their experiment with naturally bred progeny. In light of these combined results, it seems that paternal reproductive tactics have a limited effect on the growth of alevins between hatch and MAWW.

Enzymatic characteristics indicated that offspring of anadromous males had somewhat greater muscle development and organismal metabolic capacities, particularly at MAWW, than offspring of mature male parr. Tissue differences in metabolic capacities allow the use of enzyme activities to follow muscle growth. As CK is primarily localized in muscle, its activity is an excellent proxy for muscle growth, but similar information can be gleaned from the activities of LDH, CS, and CCO because they are more abundant in muscle than in the other components of the caudal portion. The enzyme activities suggest that alevins fathered by anadromous males had more extensive muscular development (CK, CCO, CS, and LDH activities in the caudal section) as well as higher whole-body aerobic (CCO and CS) and excretory (GDH) capacities during the yolk resorption period (Figs. 2, 3). To assess whether this pattern came from unequal hydration of the progeny in the two lines, we examined protein-specific activities (results not shown) and found the same differences between broodlines. Offspring of anadromous males had thus accomplished a greater conversion of vitellus protein into their own tissues at MAWW than the offspring of mature male parr. These differences between the broodlines may reflect differing energetic compromises and are compatible with the suggestion that higher metabolic demands in offspring of mature male parr restricted muscle and organ development.

Histological examination confirmed that alevins fathered by the anadromous males had somewhat more advanced muscular development at hatch, with larger cross-sectional areas of white and red muscles in alevins from the anadromous lineage (Fig. 4). The higher areas of muscle were due to the presence of large fibers, particularly in red muscle (see Figs. 5–8). In the progeny of mature parr, total cross-sectional area of red muscle was positively correlated with the number of red fibers, and the density of white fibers tended to be higher for this lineage. In Atlantic salmon (Higgins and Thorpe 1990; Johnston and McLay 1997; Johnston et al. 2000) and in rainbow trout (Weatherley et al. 1980; Valente et al. 1999), faster growth is associated with a greater recruitment of small, less differentiated muscle cells (hyperplasia). Marked recruitment of small fibers may indicate investment in future growth (Weatherley 1990). Consequently, the bioenergetic strategy of alevins fathered by mature parr may not accentuate muscle growth during yolk resorption but may favor better growth following first feeding.

The lower metabolic capacities and muscular development in progeny of mature male parr may be a consequence of higher metabolic expenditures, such as the costs of their more frequent movements (Fig. 2*C*, *inset*). Enzymatic activities indicate maximum in vitro metabolic capacities, whereas physiological rates, such as rates of activity, represent the use of these capacities and the short-term manifestation of metabolic choices. Mature male parr show higher metabolic rates (related to swimming activity), food ingestion rates, and growth rates but lower growth efficiencies than nonmature parr (Tucker and Rasmussen 1999). The higher metabolic costs are thought to reflect the occupation of more demanding, high-velocity habitats. This expensive lifestyle, combined with the investment in gamete production during sexual maturation, could lead to a greater mortality of mature compared to immature parr (Myers 1984). Offspring of mature parr showing more spontaneous movements than alevins of the anadromous lineage suggests that there is a genetic mechanism leading to differences in growth. Higher activity could help these fish to start feeding after yolk resorption. Training effects, such as those enhancing the growth of the more active dechorionated trout embryos (Ninness et al. 2006), could enhance subsequent growth. These alevins could quickly become dominant and occupy more productive river habitats where food acquisition, while costly in terms of swimming requirements, is facilitated. Consequently, progeny of mature parr would be more likely to grow faster and to mature early after first feeding (Metcalfe et al. 1995). However, a strategy depending on higher energy output to facilitate food acquisition may increase risks of mortality if first feeding occurs in an unfavorable environment.

To remain effective, such a bioenergetic strategy should not drastically affect growth during the yolk resorption period. This could explain why major size differences between alevins from the two paternal tactics during the yolk resorption period are difficult to observe. The tight energy budget of fish larvae imposes constraints that cannot easily be circumvented (reviews by Wieser 1991, 1994, 1995). Standard metabolic rate is typically high in small fishes, considerably reducing their aerobic scope. Moreover, costs of swimming and growth are maximal during the larval phase. Accordingly, from an energetic point of view, the choices offered to small fish (particularly to those depending on endogenous food reserves) are few. One choice is to support somatic growth by suppressing other energy-consuming functions. The fact that growth rate and metabolic intensity (mass-specific oxygen uptake) are not correlated in the long run argues in favor of energy reallocation for growth in alevins of chinook salmon (*Oncorhyncus tshawytscha*; Rombough 1994) and in larval cyprinids (*Rutilus rutilus* and *Chalcalburnus chalcoides mento*; Wieser et al. 1988). Our study suggests that the physiological differences between broodlines reflect decreased efficiency of energy use, potentially caused by

increased activity, in alevins fathered by mature parr, rather than reallocation of energy between growth and standard metabolic rate. Wieser (1994, p. 3) points out that "both allocation of energy and efficiency of utilization may be sufficiently variable for selection to act on them and to produce different types of adaptative lifestyles."

Few studies have examined the influence of conditional male reproductive strategies on the physiological characteristics of their offspring. We have examined this question during endogenous feeding where physiological processes take place in a "closed" system and where reallocation of energy from growth to other functions could influence organismal capacities. We showed that the paternal reproductive tactic does not change overall somatic growth between hatch and MAWW. However, all biochemical and histological differences (metabolic capacities and muscle growth) indicated somewhat greater development of muscle in offspring of anadromous males. Only the enhanced spontaneous activity by progeny of mature male parr provided a mechanism whereby future growth of these fish could be favored. We thus suggest that strategies of food acquisition and energy allocation after emergence may differentiate alevins fathered by anadromous males and by mature parr. Among alevins fathered by mature parr, heightened activity at emergence would allow the fast growth often observed after the initiation of exogenous feeding of parr destined to mature early in life. However, such a strategy could reduce muscular development during the yolk sac stage and impose a lower energetic efficiency after emergence. Comparing the growth, metabolic demands, and activity of offspring of mature male parr with those of offspring of anadromous males until their "choice" of reproductive tactic should indicate the validity of this mechanism.

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