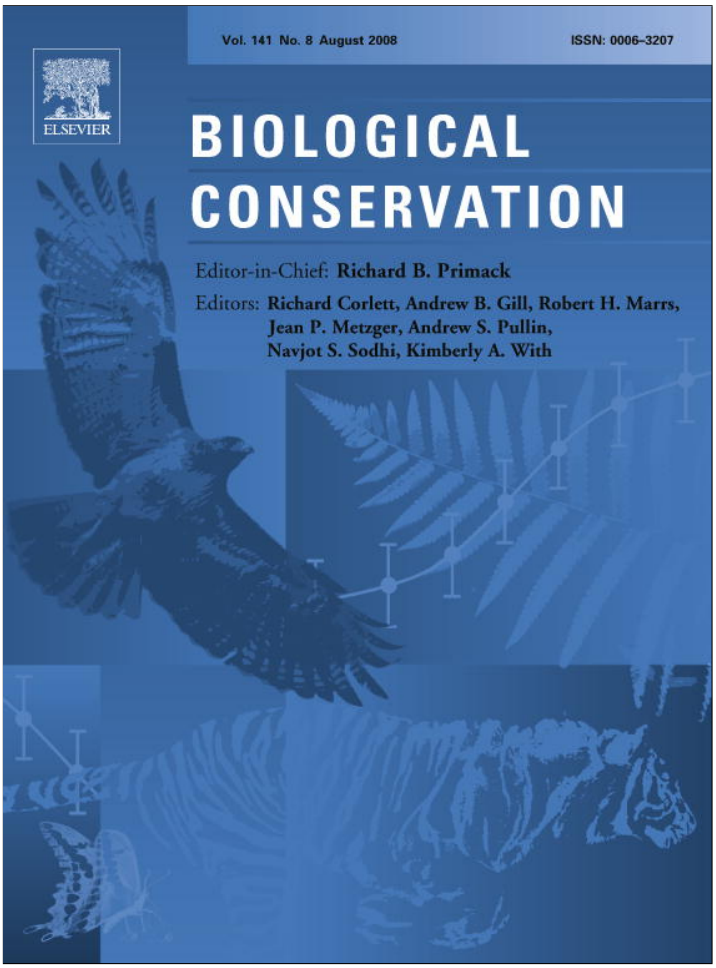

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An integrated comparison of captive-bred and wild Atlantic salmon (*Salmo salar*): Implications for supportive breeding programs

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ABSTRACT

Supportive breeding is a strategy consisting in maintaining a pool of locally-adapted wild genitors in captivity whose offspring are released in the wild at an early developmental stage. In this study, we tested the utility of this strategy in preventing phenotypic and genetic divergences between captive-bred and wild animals that could be detrimental for wild populations. Combining microsatellite analyses, morphological measurements and behavioural trials in the laboratory, we compared the progeny of Atlantic salmon (*Salmo salar*) born in captivity with individuals born in the wild. At all these levels, we found significant differences between the progeny of the two groups. Specifically, allelic frequencies significantly differed between groups, with captive-bred fish tending to be less variable with lower heterozygosity and allelic richness values. The shape of wild-born fish was also different from that of the captive-group, particularly in the depth of the head and the length of the pectoral fins. Finally, captive-bred individuals were, on average, more aggressive than wild-born fish. We demonstrated that this difference was strongly dependent upon the environment as captive-bred fish were more aggressive only when together with their wild conspecifics or with an exotic competitor, the rainbow trout (*Oncorhynchus mykiss*). Overall, our results showed that both phenotypic and genetic changes can arise even if genitors share a common brood-stock and after only a few months of rearing in a controlled environment. We conclude that the progeny produced in such supportive breeding programs does not meet the criteria necessary to ensure preserving the genetic and ecological integrity of wild populations.

1. Introduction

Captive breeding is a widely used management practice that aims to restore, conserve and/or enhance wild populations. In contrast to the potential benefits for species recovery, several authors have argued that captive breeding suffers important limitations (reviewed in Snyder et al., 1996). Particularly, captive breeding often leads to genetic, morphological and behavioural differences between captive-bred and wild populations which may pose major difficulties when attempting to

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preserve wild populations (Snyder et al., 1996; Price, 1999; Heath et al., 2003). For instance, such deficiencies may have harmful effects on wild populations (through ecological or genetic interactions) and may limit the settlement success of captive-bred animals in nature (Wang and Ryman, 2001; Ford, 2002; McGinnity et al., 2003; Theodorou and Couvet, 2004; Håkansson and Jensen, 2005; Mathews et al., 2005; Frankham, 2008). Thus, researchers have attempted to develop captive breeding strategies that aim to preserve both the genetic and phenotypic integrity of the target population (e.g. Duchesne and Bernatchez, 2002; Fiumera et al., 2004; Theodorou and Couvet, 2004).

Recently, an alternative strategy to traditional captive breeding has been developed to solve these potential problems. This strategy (called "supportive breeding"; Wang and Ryman, 2001) consists in maintaining a pool of locally-adapted wild genitors (i.e. genitors originating from the target population) in captivity whose offspring are released at an early developmental stage. In theory, such a strategy may offer several advantages. First, the genetic integrity of the population is preserved through the use of randomly caught wild breeders at each breeding season in combination with an adequate breeding design (e.g. Fiumera et al., 2000, 2004). Second, the phenotypic and genotypic differences between captive-bred and wild animals is limited by releasing first-generation captive animals at an early stage of development, thus limiting exposure to the selective pressures imposed by captivity (Salonen and Peuhkuri, 2006; Kraaijeveld-Smit et al., 2006). Although this would be context-dependent and vary according to the census size of wild populations to be restocked, a moderately high number of breeders (>20) should theoretically be maintained in captivity to avoid undesirable genetic consequences that may negatively affect fitness of wild populations (e.g. Tenhumberg et al., 2004; Theodorou and Couvet, 2004; Favé et al., 2008). Typically, such a number is not always obtainable due to logistical constraints or to the scarcity of the target population or species (Aho et al., 2006; Ramirez et al., 2006).

Evaluating the utility of captive breeding strategies is a prerequisite for future management decisions, and can be achieved through several approaches. A conservative approach is to test for genetic and/or phenotypic differences before releasing captive-bred animals into the wild (i.e., tests in the laboratory and/or in a controlled environment). To date, most studies that aim to compare captive-bred with wild animals consider captive populations that (i) were founded by genitors from non-target wild populations and/or (ii) remained captive for up to two generations (e.g. Mathews et al., 2005; Kelley et al., 2006; Salonen and Peuhkuri, 2006). Therefore, the assumption that supportive breeding designs are useful for limiting genetic and phenotypic divergences has, to our knowledge, rarely been tested.

Evaluating the success of captive-bred animals in nature and their impacts on wild populations involves assessing the genetic risks (e.g. inbreeding depression, change in allelic frequencies and/or the introduction of deleterious alleles in wild populations, Ryman et al., 1995; Ford, 2002) as well as the morphological, physiological and behavioural capabilities of captive-bred fish to survive in the wild. For instance, parameters linked to genetic diversity (e.g. heterozygosity and allelic richness) are important for populations to face environmental changes (Frankham, 2008), and traits such as body size and competitive ability determine an individual's ability to exploit and survive in natural habitats (e.g. Håkansson and Jensen, 2005; Hill et al., 2006; Kraaijeveld-Smit et al., 2006). Although many studies have only considered one or two of these components (e.g. McPhee, 2004; Hill et al., 2006; Kraaijeveld-Smit et al., 2006), the information contained in all of these components is needed to accurately forecast the success of captive-bred animals in the wild and the effects they may have on wild populations (Kraaijeveld-Smit et al., 2006).

Recent studies (Mathews et al., 2005; Kelley et al., 2006) have highlighted the importance of considering several environments when attempting to compare the behaviour of captive-bred and wild animals. For example, as density is generally higher in captivity and behaviour may vary according to density (Price, 1999; Blanchet et al., 2006; Kelley et al., 2006), one can hypothesize that captive-bred animals behave differently according to the density they encounter in the environment. Similarly, wild animals may also coexist with interspecific competitors that have been recently introduced in their habitat (i.e., exotic species). Thus, if captive-bred animals are unable to perform well (relative to their wild conspecifics) in the presence of an exotic competitor, one can hypothesize that the exotic species may limit the settlement of the captive-bred animals.

Currently, the enhancement of wild stocks of salmonid fishes using hatchery-born fish to restore or supplement wild populations is practiced worldwide, although the benefits remain uncertain (Dodson et al., 1998). The question of the utility of supportive breeding strategies is therefore particularly relevant for this group of animals. For instance, Atlantic salmon (*Salmo salar*) is a cultural and economically important species. Atlantic salmon stocks are severely declining throughout the species distribution, with some populations being considered endangered or nearly extinct (Klemetsen et al., 2003). Atlantic salmon naturally inhabit rivers of the North-Atlantic coastlines, where they spend their first two to five years of life. They then migrate to the ocean to feed and grow for one year or more and then return to their natal rivers to spawn (see Klemetsen et al., 2003 for more information). In this species, supportive breeding consists in producing fish (from locally-adapted genitors) in a hatchery and releasing them at the juvenile stage (usually within the first year after hatching) into the wild (Tessier et al., 1997; Dodson et al., 1998). Juvenile Atlantic salmon are territorial predators interacting through interference competition to defend profitable territories that are rich in food and provide refuges against predators (Klemetsen et al., 2003). In some rivers, juvenile salmon must also confront exotic competitors such as the rainbow trout (*Oncorhynchus mykiss*), an anadromous salmonid from the north-western coast of North America, representing an additional risk to declining populations (Blanchet et al., 2007). The ability of captive-bred fish to adapt to these competitive and unpredictable environments is crucial to forecast the benefits and also the genetic and ecological consequences of supportive breeding programs.

The main objective of this study was to assess the utility of a supportive breeding strategy in preventing potential diver-

gences between captive-bred and wild-born animals that are usually considered detrimental for the target population. To do so, we compared the phenotypic and genetic characteristics of the progeny of wild breeders produced in captivity and in the wild. We posit the null hypothesis that no genetic or morphological changes occurred between captive-born and wild-born Atlantic salmon sharing the same brood-stock. We also compared the behaviour of captive-born and wild-born Atlantic salmon in different competitive environments to test the null hypotheses that (i) no behavioural changes occurred between captive-born and wild-born Atlantic salmon (ii) the behaviour of captive-born animals is not density-dependent and (iii) captive-born and wild-born conspecifics behave similarly in the presence of an exotic competitor.

2. Methods

2.1. Study populations

The two groups of young-of-the-year (YOY) fish we compared were produced from adult Atlantic salmon from the river Malbaie (Québec, Canada, 47° 67′N; 70° 16′W). The Atlantic salmon population in this river was relatively small until a restoration program began in 1992. Presently, about 400–500 adults enter this river annually to spawn. A pool of genitors (i.e., 10 anadromous males and 10 females) is maintained at the provincial hatchery of Tadoussac. Each year, this pool is renewed by haphazardly capturing approximately 10 wild genitors (five anadromous males and five females) in a fish ladder installed on an insurmountable dam during the summer spawning migration. An effective breeder's pool of 20 genitors (with an equal sex-ratio) renewed at 50% each generation falls within the range theoretically predicted for preserving wild populations for a population with such census size (Duchesne and Bernatchez, 2002; Favé et al., 2008) and in the range used in other programs (Aho et al., 2006; Ramirez et al., 2006). At the hatchery, the genitors are mated and the progeny is reared in tanks. Fish densities at this hatchery are relatively low (~500 YOYs m^{-3} compared to ~2000 YOYs m^{-3} in commercial hatcheries, see McDonald et al., 1998), and water temperature is free to fluctuate naturally as the hatchery receives its water supply from a neighbouring lake. Fish are reared until the age of about four months before being released in September. Thus, the salmon population in the river Malbaie consists of wild-born and hatchery-born fish. In the downstream part of this river (i.e., below the fish ladder) a self-sustaining population of exotic rainbow trout cohabits with the Atlantic salmon.

During August 2005, before hatchery-born fish were released in the river, we sampled (by electrofishing) 250 YOY Atlantic salmon at two locations above the dam (these fish are hereafter referred to as the "wild group"). In addition, we sampled 100 YOY rainbow trout in the downstream part of the river. During the same period, we haphazardly sampled 25 YOYs from each of 10 families produced in 2005 for the supportive breeding program. These 250 fish are hereafter referred to as the "hatchery group".

We transferred all fish groups to the laboratory and raised them in separate holding tanks. They were fed *ad-libitum* with commercial fish food pellets before the beginning of the experiments. Behavioural experiments were conducted during the following winter (i.e., five months after fish capture). Mortality in the holding tank was relatively low (i.e. 10–20 fish per group) suggesting that no strong artificial selection (induced by laboratory conditions) occurred during this period.

2.2. Genetic analyses

We selected 35 fish from each experimental group ($n = 70$). Total DNA was extracted from muscle tissue using a salt extraction method described in Aljanabi and Martinez (1997). Ten nuclear microsatellite loci were amplified using polymerase chain reaction (PCR) (Table 1). PCR products were run on an ABI™ 3100 automated capillary sequencer (Applied Biosystems). Allelic sizes were scored using GENESCAN™ analysis v.3.7 and GENOYPER™ v.3.7 NT software.

2.3. Morphometric analysis

For the morphometric analysis, we selected 30 fish from each experimental group ($n = 60$). Fish were euthanized with an excess of the sedative eugenol and each was positioned on their left side on a measuring board with the lower jaw closed and caudal fin extended. Each individual was photographed using an Olympus digital camera. From each image, 16 morphological traits (Fig. 1) were measured to the nearest 0.001 mm using the free software IMAGE J (U.S. National Institute of Health, http://rsb.info.nih.gov/ij/). We focused on these traits because they have been associated with swimming performance and habitat selection (Letcher, 2003; Páez et al., 2008).

2.4. Behavioural analyses

Behavioural tests were conducted using 12 artificial channels fitted with a re-circulating water system. Each channel measured 1.90 m long, 0.30 m wide and 0.30 m deep. One pool/riffle succession was simulated using small pebbles (see Blanchet et al., 2006 for more details). Water depth and water velocity were consistent with natural habitat conditions experienced by juvenile Atlantic salmon (Klemetsen et al., 2003; Blanchet et al., 2007). Two large pebbles (7–10 cm diameter) were added in each riffle and each pool as refuges. Photoperiod was controlled with two 60-W lights above each channel at 80% and 7% of the available intensity during day, dawn, dusk, and night, respectively. Light-to-dark cycle was 9:14 h plus 30 min of dawn and of dusk. Light intensity and photoperiod were automatically set with a photoperiod monitor (SunMatch, Aquabiotech Inc.). We maintained water temperature constant at 14 ± 1 °C. Fish were fed with commercial food pellets. Daily food ration (4% of the total wet biomass per channel and per day) was dispensed at the upstream end of the channel.

The behavioural experiment involved seven competitive treatments (Table 2) designed to separate the effect of adding a given competitor from a simple density effect (see Weber and Fausch, 2003). Firstly, we tested each group independently, only varying their intraspecific densities (i.e. in treatments 1 and 3, we observed three fish per channel and in treatments 2 and 4 we observed six fish per channel).

Table 1 – Microsatellites used to compare the genetic characteristics of wild-born versus hatchery-born Atlantic salmon

Locus	A	Rx volume (μl)	Rx buffer (μl)	dNTPs (μl)	Taq (U)	Cycle (temperature in Celsius degrees)	Reference
CA 054978	5	10	1	1	0.5	2 min at 94, 35 × (30s at 94, 30s at 55, 30 s at 72), 5 min at 72	Rise et al. (2004)
CA 054565	4	10	1	1	0.5	3 min at 94, 35 × (50 s at 95, 50 s at 60, 50 s at 72), 3 min at 72	Rise et al. (2004)
Ssa 401UOS	14	10	1	1	1.5	2 min at 95, 35 × (45 s at 95, 1 s at 59, 45 s at 72), 5 min at 72	Cairney et al. (2000)
Ssa 417UOS	20	10	1	0.5	0.5	3 min at 96, 32 × (50 s at 95, 50 s at 60, 50 s at 72), 5 min at 72	Cairney et al. (2000)
SSAD 237	20	10	1	0.3	0.4	5 min at 94, 34 × (45 s at 94, 45 s at 60, 120 s at 68), 10 min at 68	King et al. (2005)
Strutta-12	11	10	1	0.3	0.3	3 min at 94, 32 × (45 s at 94, 45 s at 57, 60 s at 72), 5 min at 72	Poteaux et al. (1999)
Ssa 197	12	10	1	0.3	0.2	3 min at 94, 32 × (45 s at 94, 45 s at 57, 60 s at 72), 5 min at 72	O'Reilly et al.*
SSSOSI 417	11	10	1	0.3	0.2	3 min at 94, 32 × (45 s at 94, 45 s at 57, 60 s at 72), 5 min at 72	Slettan et al.*
SsaD 85	23	13.5	1.9	0.45	0.2	5 min at 94, 34 × (45 s at 94, 30 s at 60, 120 s at 68), 10 min at 68	Eackles and King*
SSAD 71	24	13.5	1.9	0.45	0.2	5 min at 94, 34 × (45 s at 94, 30 s at 60, 120 s at 68), 10 min at 68	King et al. (2005)

Description of the ten microsatellites markers used to compare genetic parameters between captive-born and wild-born Atlantic salmon. Number of alleles (A) is presented for each locus. The buffer comprised 10 mM Tris–HCl [pH 9.0], 1.5 mM $MgCl_2$, 0.1% Triton X-100, 50 mM KCl. The concentration of dNTPs was 10 mM each. References denoted with * correspond to loci that were directly submitted by authors to GeneBank.

Secondly, we assessed behaviour when the two salmon groups were in contact together (i.e. in treatment 5 we observed three fish of each group per channel, Table 2). Finally, we assessed behaviour when the two salmon groups were in contact with the rainbow trout (i.e. we independently observed three fish of each group together with three rainbow trout per channel in treatments 6 and 7, Table 2). Each treatment lasted five days, including a three-day acclimatisation period followed by two days of observation. Each treatment was replicated four times over a period of 24 days, with new fish used for each replicate (i.e. no fish was ever used twice).

During the experiment, fish body weight was 3.22 ± 0.92 g (mean ± SD), but it varied among replicates (one-way ANOVA, $p = 0.03$), with fish from the last replicate being bigger. However, within replicates, there were no size differences between treatments (nested ANOVA, $p = 0.21$) or among groups and/or species (nested ANOVA, $p = 0.83$). When wild and hatchery salmon were observed together, fin erosion (particularly of the pectoral fins) of hatchery fish permitted the observer to easily distinguish the two groups without specific marking.

Each channel was observed for a 10 min period in the morning (i.e. between 9 a.m. and 11 a.m.). We measured three individual behavioural variables: (1) the proportion of time spent in the pool (by default, the time spent in the riffle is the total time of observation minus the time spent in the pool) (2) the proportion of time being active (a fish was considered active when it was out of the substrate, facing the current, and propped up on its pectoral fins) and (3) number of aggressive interactions (chases, displays and nipping) initiated by each fish.

2.5. *Statistical analyses*

2.5.1. *Genetic analyses*

Individual heterozygosity (multilocus heterozygosity) levels were scored as either heterozygous (1) or homozygous (0), and these scores were averaged for all 10 loci for each individual. Differences between the two groups were then assessed using a Mann–Whitney *U*-test. To compare the allelic richness (number of alleles per locus) between groups, a correction for sample size was made using the allelic richness option in FSTAT 2.9.3 software (Goudet, 1995). We also used a Mann–Whitney *U*-test to compare the number of alleles between the two groups. Furthermore, we compared allele frequencies between the two groups using Fisher's exact test as described by Raymond and Rousset (1995a). This test was applied for each locus independently (Bonferroni corrections were applied to account for multiple comparisons) and for all the loci together. These tests were performed using GENEPOP version 3.4 (Raymond and Rousset, 1995b). Finally, we tested whether bottleneck (i.e., a drastic reduction in the number of effective breeders) effects occurred in each of the two groups. Indeed, because of the small wild population size in river Malbaie, it is possible that this population suffered a bottleneck well before the restoration program was initiated. In the captive-group, a bottleneck is expected as only a small portion of the total pool of genitors is used to produce progeny. After a bottleneck event, the allelic diversity is expected to decline faster than heterozygosity. Thus, the observed heterozygosity

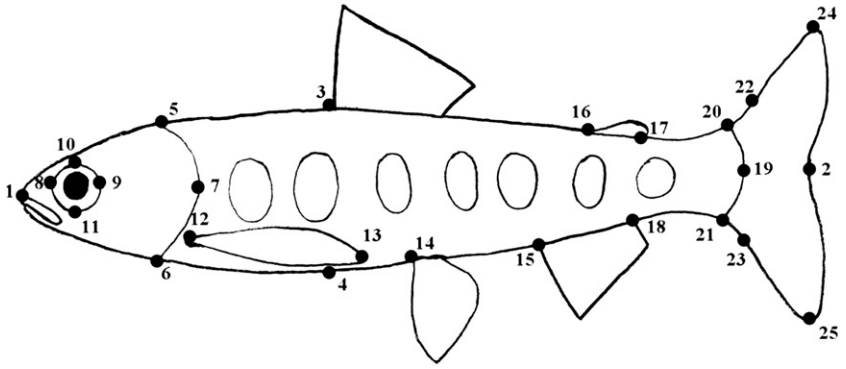

Fig. 1 – Landmarks used for morphological measurements. 1–2 Fork length (F_L), 3–4 maximum body depth (B_D), 1–7 head length (H_L), 5–6 head depth (H_D), 8–9 orbital length (O_L), 10–11 orbital depth (O_D), 1–5 distance between tip of the snout and highest head height (SH_L), 12–13 pectoral fin length (PC_L), 1–3 predorsal length (PD_L), 1–14 prepelvic length (PP_L), 3–15 distance between origins of the dorsal and anal fins (ODA_{FL}), 3–16 distance between the origins of the dorsal and adipose fins ($ODAD_{FL}$), 17–18 distance between insertions of the adipose and anal fins (OAA_{FL}), 18–19 caudal peduncle length (DP_L), 20–21 caudal peduncle depth (CP_D), 22–23 minimum caudal fin height ($MinC_{FH}$), 24–25 maximum caudal fin height ($MaxC_{FH}$).

Table 2 – Design of the experiment used to compare the behaviours of wild-born versus hatchery-born Atlantic salmon

	Wild-born salmon	Hatchery-born salmon	Rainbow trout
Low intraspecific W	3	–	–
High intraspecific W	6	–	–
Low intraspecific H	–	3	–
High intraspecific H	–	6	–
Mixed intraspecific	3	3	–
Interspecific W	3	–	3
Interspecific H	–	3	3

Experimental design used to evaluate the effects of intraspecific and interspecific competition on the behaviour of hatchery-born and wild-born Atlantic salmon. The number of fish introduced in each treatment (seven treatments) is indicated. "W" means wild-born fish and "H" means hatchery-born fish. Each treatment was replicated four times ($n = 4$) and new fish were used for each replicate.

should be larger than the heterozygosity expected from the observed allele number at mutation-drift equilibrium (Cornuet and Luikart, 1996). We tested this assumption using the *BOTTLENECK* software (Cornuet and Luikart, 1996). This software used sign test to compare the observed number of loci with heterozygosity excess to the number expected under the mutation-drift equilibrium (Cornuet and Luikart, 1996).

2.5.2. *Morphometric analyses*

We used ANCOVAs (analyses of covariance) to asses shape differences between the two groups of fish while controlling for the allometric effect of body size on each morphological trait. We first computed a multivariate ANCOVA (MANCOVA) with the total body length of each fish as the covariate and the origin of the fish as categorical predictor. The resulting two-term interaction was also included to test for allometric differences between the two groups of fish. If the MANCOVA was significant, we then used a univariate ANCOVA to assess how each trait independently differed between each group. Significant p-values were corrected for multiple comparison using a Bonferroni procedure (so that the acceptable significance level was reduced to $\alpha < 0.05/16 = 0.003$).

2.5.3. *Behavioural analyses*

A two-way MANCOVA was computed to test whether the general behaviour of the fish changed according to origin (wild or hatchery) and competitive treatments (see Table 2). We used the averaged body length of Atlantic salmon in each replicate and for each group as the covariate. All two-term interactions were considered in this analysis. If the MANCOVA was significant, we performed three independent ANCOVAs to compare (i) the aggression rate, (ii) the time spent in the pool and (iii) the time spent being active. The aggression rate was $\log(x + 1)$ transformed and the two other behaviours were arcsine transformed to meet the assumption of normality and homoscedasticity. Initially, a "trial" factor was included in the model to account for possible temporal effects. As it was not significant, this term was excluded from the final models.

All statistical analyses were performed using R version 2.2.1 (R Development Core Team, 2005).

3. Results

3.1. *Genetic analyses*

When all loci were pooled, allele frequencies significantly varied between the two groups (Fisher exact test, $\chi^2 = +\infty$, $p = 0.000$, Table 3A). Seven out of the ten loci we compared showed different frequencies between the two groups after the Bonferroni correction (Table 3A). As illustrated in Fig. 2, some of these loci showed marked differences, with each group being characterized by specific patterns of allelic frequencies and sometimes by private alleles with high frequencies. The allele frequencies for all the ten loci used in this experiment are available upon request to the corresponding author. We also found that these frequency differences translated into a trend whereby both individual heterozygosity and allelic richness tended to be lower in the hatchery than in the wild group (Table 3B). These differences, however, were

Table 3 – Genetic parameters of wild-born versus hatchery-born Atlantic salmon

		Fisher exact test
(A) Allelic frequencies		
CA054978		**0.001 ± 0.000**
CA 054565		0.198 ± 0.002
Ssa 401UOS		**0.000 ± 0.000**
Ssa 417UOS		**0.000 ± 0.000**
SSAD 237		**0.000 ± 0.000**
Strutta-12		**0.000 ± 0.000**
Ssa 197		0.031 ± 0.001
SSSOSl 417		0.053 ± 0.002
SsaD 85		**0.000 ± 0.000**
SSAD 71		**0.000 ± 0.000**
All loci		**0.000**
	Hatchery fish	Wild fish
(B) Genetic diversity		
Allelic richness	8.61 ± 1.00	14.87 ± 4.08
Multilocus heterozygosity	0.75 ± 0.02	0.81 ± 0.02

Results of several tests used to compare genetic parameters between hatchery-born ("Hatchery fish") and wild-born ("Wild fish") Atlantic salmon. Table (A) – results (p-value ± SD) of Fisher exact tests used to compare the allelic frequencies, for each marker independently and for all the loci combined, between the hatchery and wild groups. Table (B) – genetic diversity (mean ± S.E. for allelic richness and multilocus heterozygosity respectively) for both groups of salmon. Bold p-values are significant after Bonferonni corrections.

marginally non-significant (multilocus heterozygosity; Mann–Whitney U-test, $z = -1.87$, $p = 0.06$ and allelic richness; $z = -1.68$, $p = 0.09$).

The number of loci showing heterozygosity excess was significantly higher than what expected by chance for the captive-born group (10 out of the 10 loci; sign test, $p = 0.006$), indicating that the small number of genitors used in the captive pool created a recent genetic bottleneck. In contrast, the number of loci showing heterozygosity excess did not differ significantly from what was expected by chance in the wild-born group (7 out of the 10 loci; sign test, $p = 0.373$).

3.2. *Morphometric analyses*

The shape of wild-born fish significantly differed from that of the hatchery group (MANCOVA, effect of the fish body length, Wilks' λ (1, 16) = 0.008, $p < 0.001$; effect of the origin of the fish, Wilks' λ (1, 16) = 0.274, $p < 0.001$; interaction term, Wilks' λ (1, 16) = 0.550, $p = 0.055$). At the univariate level, 6 out of the 16 traits we measured significantly differed between the two groups (Table 4). When ranked according to the F value, the greatest differences between the two groups involved the depth of the head and the length of the pectoral fins (Table 4). Specifically, for a given length, wild fish had the deepest heads and the longest pectoral fins. Other functionally important traits, which remained larger in the wild group following size correction, were the maximum span of the caudal fin and the depth of the caudal peduncle (see Table 4). It is worth noting that for one trait (the orbital depth, see Table 4) we detected a significant interaction term ($p < 0.003$) indicating that the allometric relationship of this trait differed between the two groups. Specifically, the slope of the relationship between the orbital depth and the body length of Atlantic salmon was higher in the hatchery group (slope = 0.074) than in the wild group (slope = 0.039).

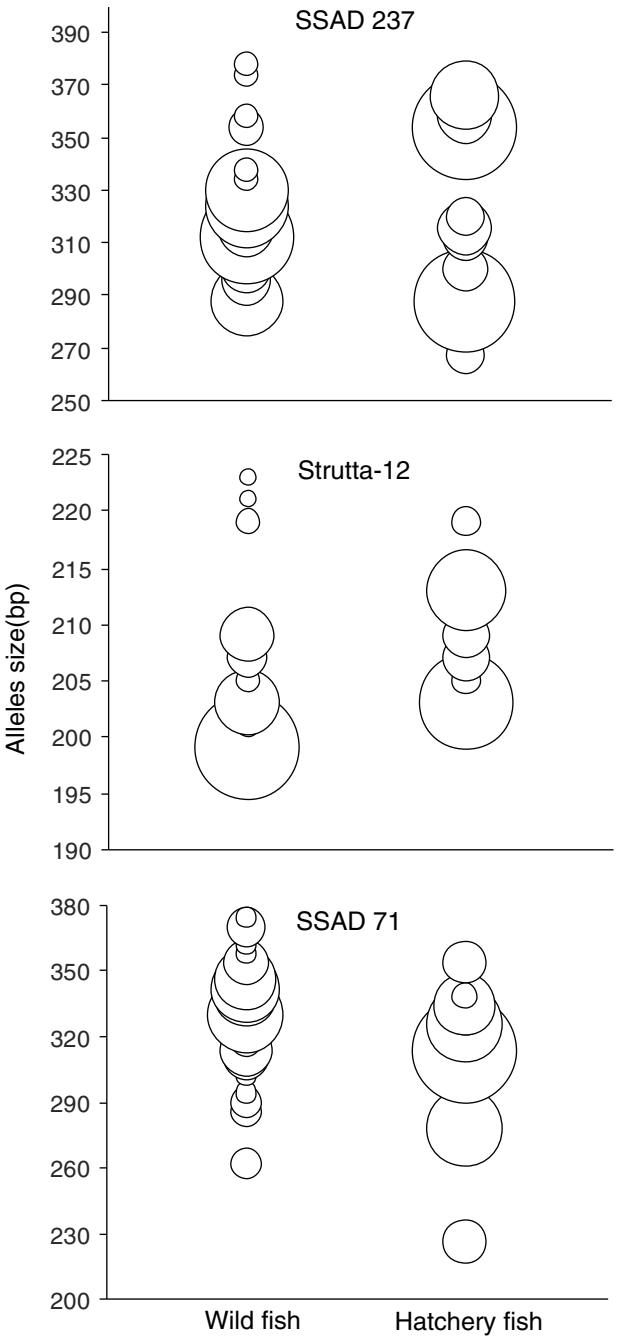

Fig. 2 – Allelic frequencies of wild-born versus hatchery-born Atlantic salmon. Schematic illustration of relative allelic frequencies of three out of seven loci (SSAD 237, Strutta-12 and SSAD 71, see Table 3) that showed significant frequency differences between hatchery-born ("Hatchery fish") and wild-born ("Wild fish") Atlantic salmon. These three loci were chosen for illustration as they displayed the highest differences between groups. Allele frequencies for all the loci are available upon request to the corresponding author.

Table 4 – Morphological characteristics of wild-born versus hatchery-born Atlantic salmon

Response variable	Hatchery fish	Wild fish	*F* value	*p*-value
Pectoral fin length	−0.058 (±0.020)	0.057 (±0.008)	53.29	**0.000**
Head depth	−0.007 (±0.003)	0.007 (±0.002)	22.18	**0.000**
Head length	0.005 (±0.003)	−0.005 (±0.003)	17.43	**0.000**
Maximum caudal fin height	−0.014 (±0.006)	0.013 (±0.008)	12.05	**0.001**
Minimum caudal fin height	−0.007 (±0.005)	0.007 (±0.005)	10.56	**0.002**
Caudal peduncle depth	−0.005 (±0.004)	0.005 (±0.003)	8.77	**0.003**
Distance origins of the dorsal and anal fins	0.004 (±0.003)	−0.004 (±0.003)	5.08	0.028
Prepelvic length	−0.002 (±0.002)	0.002 (±0.001)	4.46	0.039
Orbital depth	−0.004 (±0.005)	0.004 (±0.004)	2.30	0.134*
Predorsal length	−0.006 (±0.011)	0.006 (±0.003)	1.85	0.179
Caudal peduncle length	0.004 (±0.009)	−0.004 (±0.005)	0.99	0.330
Distance insertions of the adipose and anal fins	0.002 (±0.005)	−0.002 (±0.005)	0.49	0.483
Maximum body depth	0.002 (±0.003)	−0.002 (±0.007)	0.27	0.601
Distance outer tip of the snout/highest head height	0.001 (±0.006)	−0.001 (±0.005)	0.19	0.657
Orbital length	−0.001 (±0.005)	0.001 (±0.004)	0.14	0.701
Distance the origins of the dorsal and adipose fins	0.001 (±0.002)	−0.001 (±0.002)	0.14	0.705

Results of ANCOVAs used to compare each morphological trait (see Fig. 1 for a description of the traits) independently between hatchery-born ("Hatchery fish") and wild-born ("Wild fish") Atlantic salmon. The means corrected for fish body length (±SE) for each trait and each group are given for comparison. Bold *p*-values are significant after Bonferoni corrections. A star (*) indicated trait(s) for which a significant interaction term between fish body length and origin of the fish was detected.

3.3. *Behavioural analyses*

The general behaviour differed significantly between wild and hatchery fish and also between competitive treatments (Table 5A). When all the behaviours were considered together in a single analysis, differences between wild and hatchery fish were consistent across competitive treatments as the interaction between "groups" and "competitive treatments" was not significant (Table 5A). Univariate analyses revealed different patterns according to the behaviour we considered (Table 5B). For the two variables related to habitat use (time spent in the pool and time spent in active swimming), we found significant differences among the competitive treatments but no differences between hatchery-born and wild-born fish (Table 5B). For both groups, fish were found more often in pools and were more active when rainbow trout was also present in the channels (Fig. 3a and b). Concerning the aggression rate, we found a significant effect of individual

Table 5 – Statistical comparison of the behaviours of wild-born versus hatchery-born Atlantic salmon

Source of variation		d.f.	Wilks' λ	*p*-value
(A) *Multivariate analysis*				
Body length		3,19	0.79	0.248
Groups		3,19	0.50	**0.007**
Competitive treatments		9,19	0.36	**0.027**
Body length * groups		3,19	0.91	0.627
Body length * competitive treatments		9,19	0.67	0.602
Groups * competitive treatments		9,19	0.65	0.534
		Response variables		
		Time spent in the pool	Time spent being active	Aggression rate
(B) *Univariate analyses*				
Body length	$F_{1,19}$	0.17	2.51	4.83
	P-value	0.678	0.129	**0.043**
Groups	$F_{1,19}$	1.08	0.01	31.92
	P-value	0.309	0.907	**<0.001**
Competitive treatments	$F_{3,19}$	4.39	3.14	2.33
	P-value	**0.016**	**0.049**	0.112
Body length * groups	$F_{1,19}$	0.39	0.97	1.25
	P-value	0.539	0.335	0.280
Body length * competitive treatments	$F_{3,19}$	0.61	0.44	2.29
	P-value	0.610	0.726	0.116
Groups * competitive treatments	$F_{3,19}$	0.37	0.17	3.82
	P-value	0.777	0.915	**0.011**

Multivariate (A) and univariate (B) analyses of variance used to compare the behavioural responses (time spent in the pool, time spent being active and aggressive rate) between hatchery-bred and wild Atlantic salmon under different competitive treatments. Analysis of variance included the fixed effects of groups and competitive treatments. Significant *p*-values ($p < 0.05$) are in bold.

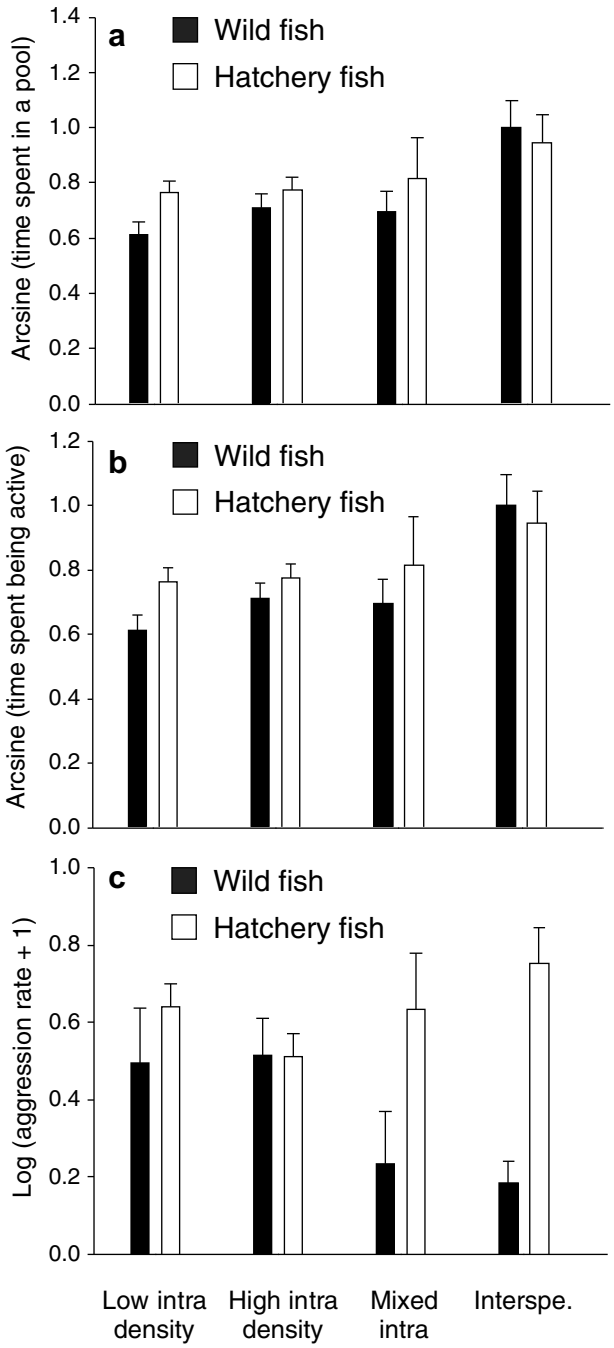

Fig. 3 – Behavioural repertory of wild-born versus hatchery-born Atlantic salmon. Patterns of behavioural responses [(a) time spent in a pool, (b) time spent being active and (c) aggression rate] of hatchery-born ("Hatchery fish", white bars) and wild-born ("Wild fish", black bars) Atlantic salmon when reared under four different competitive treatments see Table 2 for a description of the treatments. Data are the mean (±S.E.). Each treatment was replicated four times (n = 4) and new fish were used for each replicate.

body length (Table 5B) with bigger fish being on average more aggressive (results not shown). Irrespectively of individual body length, we found a significant interaction between groups and competitive treatments (Table 5B). This interaction indicated that hatchery fish differed from wild fish but only in some of the treatments (Table 5B, Fig. 3c). Particularly, hatchery fish were more aggressive than wild fish only in presence of their wild conspecifics or with the rainbow trout (Fig. 3c).

4. Discussion

Here, we applied an integrated approach to assess phenotypic and genetic differences between wild- and captive-born salmon in the context of a supportive breeding program. We highlighted significant genetic, morphological, and behavioural differences between the progeny of captive-bred and wild-born Atlantic salmon. Moreover, we showed that both phenotypic and genetic changes can arise even if the genitors share the same brood-stock and after only a few months of rearing in a controlled environment.

Taken individually, our results are consistent with previous observations concerning the effects of captivity on the genetic and phenotypic integrity of wild species. For instance, we found that the captive-bred salmon (i) had allelic frequencies that significantly varied from the wild-born progeny and (ii) tended to have lower genetic variability. Thus, even if the genitors share the same brood-stock, the single breeding event that separated the two experimental groups impacted the genetic integrity of the target wild population. The observation that observed heterozygosity was larger than the heterozygosity expected from the observed allele number at mutation-drift equilibrium in the captive-group probably results from a bottleneck inherent in the low number of genitors used in captivity (Ryman et al., 1995; Tessier et al., 1997; Kraaijeveld-Smit et al., 2006). It is worth noting that before the restoration program began in river Malbaie, the population size was relatively small, as for most populations that are endangered or exploited (Smith and Bernatchez, 2008). However, it did not seem that this low population size was sufficient to induce a detectable bottleneck in the wild-born group. These results suggest that the number of breeders (or refreshment rate) used in supportive design should be carefully estimated to avoid undesirable genetic effects.

Similarly, significant morphological and allometric differences were detected between captive-bred and wild-born salmon, with the strongest changes observed for fin lengths and other aspects of body shape, that are functionally important for swimming. These results concur with those obtained for other animal species (e.g. Håkansson and Jensen, 2005) and particularly with those obtained for salmonids (Kostow, 2004; Dahl et al., 2006, reviewed in Weber and Fausch, 2003). As demonstrated by Kostow (2004) in rainbow trout, the morphological differences seen in captive-bred individuals were directly associated with a higher mortality rate in the wild. This link has been interpreted as a consequence of the reduced ability of captive animals to occupy favourable habitats (Weber and Fausch, 2003). Although we failed to detect significant differences in habitat use by captive-bred and wild-born animals in our laboratory setting, an association between morphology and habitat use seems highly plausible in the wild. Indeed, morphological traits such as long paired fins and a large caudal peduncle and caudal fin facilitate the

exploitation of habitats such as high current velocity zones, at lower energetic costs (Arnold et al., 1991; Letcher, 2003; Páez et al., 2008).

Changes in behavioural characteristics such as activity or aggression rate have also been associated with poor survival rate of captive fish in the wild (i.e. a high predation rate, see Biro et al., 2004). Here, in accordance with many other studies, we found that captive-bred animals were on average more aggressive than wild-born conspecifics. This suggests that the protocol used to raise fish under hatchery conditions induces behaviours that may be maladapted for surviving in the wild or that may be detrimental for wild populations (Biro et al., 2004; McPhee, 2004; Mathews et al., 2005; Kelley et al., 2006).

We also demonstrated that such a behavioural difference between groups was highly dependent on the social environment (i.e. density and type of competitors). Indeed, captive-bred fish were more aggressive only when together with their wild-born conspecifics or when together with rainbow trout. It is worth noting that this difference was due to a concomitant decrease and increase in aggression of the wild and hatchery group, respectively. This also indicates that wild-born salmon strongly modify their natural behaviour (i.e. in comparison to the purely intraspecific treatments) when facing captive-bred salmon and/or rainbow trout. This behavioural plasticity has several important implications for supportive breeding programs. First, by being more aggressive when together with wild-born salmon, captive-raised animals could exert a new selective pressure on wild-born individuals, as suggested by Blanchet et al. (2007) in the case of competition between exotic and native salmonids. Second, the behavioural response of Atlantic salmon differed between groups when facing an exotic competitor. This result suggests that the captive environment can change behavioural characteristics that are relevant to biological interactions such as interspecific competition.

Taken as a whole, our results provide insight into the mechanisms underlying the different phenotypic changes we documented. In the absence of genetic data, many authors have hypothesized that because of a common brood-stock (hence a common genetic background) phenotypic plasticity predominantly determines phenotypic differences between captive-bred and wild animals (Kostow, 2004; Dahl et al., 2006). This hypothesis is particularly attractive when considering animals that phenotypically diverge within a single generation of captivity. In our case, phenotypic plasticity probably contributes in part to the phenotypic differentiation we observed between groups. However, since we observed genetic differences between both groups, we can also suggest that these phenotypic differences also result from the phenotypic expression of functional alleles present in the wild-born group that are not expressed in captivity (or vice versa) (Wedekind, 2002; Heath et al., 2003). Thus, if genetic factors contribute to the differentiation of captive-bred and wild-born animals, one can expect potential detrimental effects for wild populations in the case of interbreeding between captive-bred and wild-born animals (Araki et al., 2007b; Roberge et al., 2008). For instance, Araki et al. (2007b) used pedigree analyses to demonstrate that in the rainbow trout the genetic effects of domestication strongly reduced subsequent reproductive capabilities when captive fish were moved to natural environments and when they interbred with wild conspecifics. This implies that further studies should be specifically designed to elucidate the relative importance of genetic *versus* environmental factors (and their interaction) on the phenotypic differentiation of captive-bred and wild animals during early ontogeny.

The number of breeding events in captivity separating our two groups of fish was difficult to evaluate. Indeed, the wild-born fish we used here might have originated from captive-reared fish previously released in the River Malbaie. Similarly, the genitors that were used by the hatchery to produce the captive-bred fish might themselves have originated from captive-bred fish, signifying in this case that more than a single breeding event could have separated purely-wild fish from captive-bred fish. Araki et al. (2007b) recently demonstrated that the effect of captivity on fitness was cumulative with a fitness decline of ~37.5% per captive-reared generation. Thus, the phenotypic and genetic differences we report might directly reflect this cumulative effect of captivity. These differences are expected to increase if measures are not taken to ensure that genitors used in captivity have not been themselves bred in captivity.

5. Conclusions and implications

We showed that using genitors from a "local" brood-stock and a limited rearing period in captivity did not prevent the morphological, behavioural and genetic divergences that have commonly been identified as detrimental for target populations. Therefore, the progeny produced in such supportive breeding programs does not meet the criteria necessary to ensure preserving the genetic and ecological integrity of wild populations. In view of these results and following the precautionary principle, we argue that such supportive breeding programs cannot be considered as acceptable conservation strategies, at least in their present form. However, such programs could easily be improved. For instance, the first aim of these programs should be maintaining the genetic diversity and allelic frequencies of the native population rather than maximizing production. To do so, the genetic structure of the native population must be well known, the census size of the captive breeders must be adequately calculated and frequently refreshed, and factorial breeding designs should be favoured (Fiumera et al., 2000, 2004; Duchesne and Bernatchez, 2002; Wedekind, 2002). Also, it has recently been demonstrated that equalizing milt volume of males reduces the loss of genetic variation in a captive population (Wedekind et al., 2007). Efforts should also be devoted to avoid using genitors that are themselves issued from captive brood-stock and hence to limit the cumulative effects of captivity (Araki et al., 2007b). Second, selection and/or plasticity acting during captivity must be reduced to avoid phenotypic differentiation. This can be achieved by reducing the captive period. Metcalfe et al. (2003) proposed to limit the period of captivity to egg production with release at the egg stage. Another possibility, albeit more costly, would be to rear captive juveniles in more natural conditions by increasing the physical heterogeneity of the captive habitat or by building artificial nursery habitat (see Berejikian et al., 2000). Once this has been achieved,

short-term and long-term assessment of the costs (both ecological and economical) and benefits of supportive programs can be performed in the wild through the use of parentage assignment analyses, capture-mark-recapture programs, large-scale experiments and/or meta-analyses (e.g. Tessier et al., 1997; Hansen, 2002; Brown et al., 2006; Adamski and Witkowski, 2007; Araki et al., 2007a, b).

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