

REPORT

Alternative male life-history tactics as potential vehicles for speeding introgression of farm salmon traits into wild populations

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Abstract

Releases of cultured organisms, such as farm Atlantic salmon (*Salmo salar* L.), threaten native biodiversity and the integrity of natural communities. Salmon escaping from sea farms, however, have relatively poor reproductive success, suggesting that the rate of spread of domesticated traits may be reduced. We now compare the relative reproductive success of males that mature precociously in freshwater (parr) and find that those of farm origin have higher breeding and fertilization success than wild and hybrid individuals. Specifically, hybrid parr had 57% and wild parr 25% the success of farm parr. Early maturing males could thus be important vehicles promoting introgression of domesticated and/or non-native traits into wild populations and ultimately have long-term impact on the genetic integrity of native populations.

Keywords

Cultured organisms, introgression, microsatellites, precocious parr, reproductive success, salmonids.

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INTRODUCTION

In many ecosystems, native biodiversity and the integrity of natural communities is threatened by intentional and unintentional releases of cultured organisms (IUCN 1997). This is particularly the case in aquatic ecosystems, where unintentional releases of artificially produced fishes are common and are raising concerns (reviewed in Hindar *et al.* 1991; Fleming 1995; Youngson *et al.* 2001). Namely, the recent exponential increase in the farming of Atlantic salmon (*Salmo salar* L.) (reviewed in Gross 1998) has resulted in the escape of large numbers of fish annually, many of which enter the rivers of native salmon populations (Hansen *et al.* 1991; Youngson *et al.* 1997). Potential ecological problems include modifications of density-dependent processes and competitive interactions, which can affect food

availability, growth and survival (e.g. Einum & Fleming 1997; McGinnity *et al.* 1997; Fleming *et al.* 2000). Furthermore, farm fish may differ genetically from wild fish because of their non-indigenous origin, and random genetic change (e.g. founder effects and genetic drift) and altered selective regime within culture (e.g. Youngson *et al.* 1991; Fleming & Einum 1997; Mjølnerød *et al.* 1997; Fleming *et al.* 2002). Moreover, gene flow between the two groups may disrupt local adaptations (reviewed in Taylor 1991) and reduce genetic variability through homogenization, thus threatening native populations already in decline (Kellogg 1999).

The manifestation of many of these interactions depends on the reproductive behaviour and performance of farm fish in wild salmon populations. Escaped farm fish are capable of reproducing and hybridizing with wild fish (Webb *et al.* 1991; Crozier 1993; Clifford *et al.* 1998), but are constrained

by inferior competitive and breeding behaviour (Fleming *et al.* 1996). Recently, Fleming *et al.* (2000) quantified the lifetime reproductive success (adult to adult) and interactions resulting from farm salmon (reared to maturity) invading a native population. They found farm fish to have 16% the lifetime reproductive success of native salmon and, given current levels of escape (Fiske *et al.* 2001), concluded that the potential impact on native populations from genetic introgression (i.e. disruption of local adaptations and reduction of genetic diversity) may be significant.

However, the potential for genetic introgression beyond the first generation, which will determine long-term impacts, is unknown. Fertilization by early maturing males could be a principal route by which such introgression occurs. Atlantic salmon show such an alternative male reproductive phenotype; in addition to *anadromous* males, which like females migrate to sea before maturing, there is frequently a significant component of early maturing male *parr* within populations (Hutchings & Jones 1998). These males reach maturity while still in fresh water, often at less than a hundredth the weight of anadromous fish and as early as their first year of life (Fleming 1996). The coexistence of two male phenotypes is thought to represent alternative tactics within a single conditional strategy (Bohlin *et al.* 1990; Hutchings & Myers 1994), the equilibrium of which appears to be determined by the combined effects of frequency- and condition-dependent selection (Gross 1996; Gross & Repka 1998). Both male phenotypes compete for access to ovipositing females, who alone construct the gravel nests for their eggs. However, unlike anadromous males, mature male *parr* neither court females nor exhibit well-developed secondary sexual traits for fighting, but rather attempt to sneak fertilizations. They can be very efficient in doing so, fertilizing up to 40% of the eggs within nests (e.g. Hutchings & Myers 1988; Thomaz *et al.* 1997; Garant *et al.* 2002) and having considerable genetic contribution within natural populations (Jordan & Youngson 1992; Taggart *et al.* 2001). Early maturity increases survival prospects to breed by reducing the years of mortality prior to maturation and the mortality associated with migration and, it also decreases generation time. A potentially high reproductive success and a shorter generation time may thus speed genetic introgression of farm traits into wild populations. Moreover, the innately higher levels of aggression and growth in hybrid and farm relative to wild *parr* (Einum & Fleming 1997; Fleming & Einum 1997; McGinnity *et al.* 1997; Fleming *et al.* 2000, 2002) may provide them with a competitive advantage during such breeding competition, and thus increase the potential for genetic introgression.

The objectives of this study were to quantify the relative reproductive performance of farm, hybrid and wild *parr* over a breeding season within replicate, experimental populations of anadromous Atlantic salmon and to document *parr*

behaviour, potential participation, and fertilization success at individual spawning events. In doing so, we provide the first estimates of effective mating and fertilization success of early maturing males of farm and hybrid origin under near-natural spawning conditions. It was hypothesized that the differences in reproductive success among *parr* would be less, and potentially contrary to that observed previously between recently escaped farm and wild anadromous fish (Fleming *et al.* 1996, 2000), which more likely reflected, in part, the environmental effects of captive rearing. We used microsatellite DNA analyses to quantify the breeding success and to establish the relationship between behaviour and fertilization success. Finally, by comparing the farm strain to fish from the wild population that principally founded the strain (reared under common hatchery conditions), the potential differences in performance observed may be ascribed mainly to domestication (i.e. evolutionary changes due to intentional and unintentional artificial selection).

MATERIAL AND METHODS

Study site

The experiment was conducted at the Norwegian Institute for Nature Research (NINA) Station at Ims, Norway (58°57'N, 5°58'E). We used two circular stream arenas designed to simulate natural breeding conditions, including gravel substrate (47 m²) and water velocity (arenas 1 and 2 as described in Fleming *et al.* 1996). An observation tower situated ca. 3 m above the ground overlooked each of the arenas, and floodlights adjusted by dimmer switches provided dim light for night-time observation. A grid of strings (1 × 1 m) was suspended over each arena to help in locating and recording fish and nest positions.

Biological material and experimental design

The mature male *parr* used in the experiments were derived from 18 single male–female crosses conducted in 1999 to create six families of each of wild, farm and hybrid origin fish. Seventh-generation farm adults from broodline 1 (*sensu* Gjedrem *et al.* 1991) of Norway's national breeding programme, AquaGen, and adults of the River Namsen, the principal founder population of the farm strain, were used to create the crosses. Fish of the River Namsen originally constituted 11% of the farm broodline, but by the fourth generation of selection the constitution had increased to 70% (Gjedrem *et al.* 1991) and it is unlikely that this trend stopped (Fleming & Einum 1997), such that the present farm broodline is almost solely Namsen origin. The 18 families were created and reared from eggs until the experiments (fall 2000) under common hatchery conditions at the NINA station, with rotation among tanks. Twenty

mature (identified by gently expressing of milt) age-1 male parr from each group were anaesthetized with chlorobutanol, measured for length (tip of snout to fork in tail), weighed, photographed and a small clip of adipose fin taken (placed in 95% ethanol for later genetic analysis). They were then externally tagged below the dorsal fin with a 3 mm-white bead for group identification and internally with a passive integrated transponder (PIT) tag for individual identification. Ten mature male parr of each group, matched for size among groups, were then released into each of the two arenas on 2 November 2000, after having had at least 1 day of recovery from tagging. There was no initial difference in length (mean \pm SD = 11.4 ± 1.4 cm) or weight (20.3 ± 7.7 g) of parr between the two arenas or among parr origin within each arena (ANOVA, $P > 0.60$). Similarly, there was no difference in the condition factor ($K = \text{weight}/\text{length}^3$) between arenas ($F = 1.0$, $P = 0.32$). However, within arenas farm parr had lower condition factors than hybrid and wild parr ($P < 0.001$), which in turn had lower condition factors than hybrids ($P = 0.01$) (Scheffé post-hoc comparisons of mean). A farm parr was added to arena 2 on 1 December to maintain densities, because one had been lost through the water outlet.

The anadromous adults used in the experiments were caught at a fish trap located 100 m above the outlet of the River Imsa, close to the NINA station. On 4 November, 24 anadromous females (mean weight \pm SD = 1940 ± 602 g) and 24 anadromous males (1801 ± 716 g) were anaesthetized, measured, weighed, photographed, a small clip of adipose fin taken and secured in 95% ethanol, and tagged below the dorsal fin with uniquely labelled 3.4-cm diameter disc tags. Twelve males and 12 females, matched for size between the arenas (ANOVA, $P = 0.645$), were released into each arena.

Behavioural analysis

Behavioural data were collected throughout the breeding season from 4 November to 19 December. Nest building and spawning activities were recorded 24 h day⁻¹ using overhead surveillance cameras and underwater cameras positioned in the stream near nest sites. Camera signals were fed to video cassette recorders in the observation tower. Videos were used to record spawning participation and male and female identity, and behaviour around the nests. In addition, for 19 of the nests individual parr behaviour was recorded prior, during and following spawning using a circular PIT detector, which was deployed during the early stages of female nest construction (as described in Armstrong *et al.* 2001). The detector recorded the passage of parr in and out of the nest, and was synchronized with the video allowing for individual parr identification. Each nest location was positioned

according to their grid location. On 19 December, all surviving fish were killed, total weight was measured and the presence of wounds were recorded. In February 2001, nests were excavated according to their recorded grid location, and eggs of 30 selected nests (15 in each arena) were put in 95% ethanol for genetic analysis. Nests were chosen to reflect: (i) the distribution of the numbers of parr present at spawnings (we used nests in which three parr or fewer were present because this was representative; 89% of spawnings), (ii) the observed frequency of attendance by parr of each origin, and (iii) the different females in each arena. We used one to five nests per female (one nest = one female; two nests = four females; three nests = two females; four nests = one female; five nests = one female).

Genetic analysis

Microsatellite DNA analyses were used to establish parental identity of eyed-eggs in selected nests. Total DNA extraction was performed from the tissue of the developing embryo removed from inside the egg using Qiagen micro-column DNEASY extraction kit (QIAGEN GmbH, Hilden, Germany). Microsatellite polymorphism analysis of six loci (*Ssa 85*, *Ssa171*, *Ssa197*, *Ssa202*, O'Reilly *et al.* 1996; *SSOSL85*, *SSOSL117*, Slettan *et al.* 1995) using fluorescent labelling was performed as detailed in Garant *et al.* (2000). Maternal and paternal identity was assigned by comparing the allelic identity of the potential parents with that of their offspring. We analysed 60 offspring per nest (or all if < 60), for a total of 1305 offspring which is sufficient with the microsatellite analyses to establish proportion of paternity attributable to parr (see DeWoody *et al.* 2000), given that the anadromous fish identities were also ascertainable by videos.

Statistical analysis

Data that did not meet the requirements for parametric analyses, even after log or square root transformation, were analysed by non-parametric tests with Statistica 5.5 (Statsoft 1999). All multiple comparisons were adjusted using sequential Bonferonni procedures (Rice 1989). For the spawnings involving parr (66%) and where group identity could be ascertained using the external marks (92%), the percentage attended by parr of wild, farm and hybrid origin, regardless of the number in attendance, was quantified. We also calculated the mean number of parr of each origin attending these spawnings.

Growth for each parr (G) was calculated as

$$G = 100(\ln W_2 - \ln W_1)d^{-1}$$

where W_2 and W_1 are final and initial weight of parr (in grams), respectively and d is the length of the experiment

in days. Differences between arenas and among origins were analysed by ANCOVA (Statsoft 1999) using initial weight as a covariate.

Fertilization success was measured as the proportion of eggs fertilized in each nest by each parr. As we did not analyse all nests from all the spawning events, no comparisons could be made at the individual level concerning fertilization success, but comparisons were made at the group level. We also measured fertilization efficiency, i.e. the proportion of live embryos sampled within a nest that each parr fertilized. At the group level, this was analysed in two ways: (1) comparing the number of spawnings that parr of a given origin attended relative to the number in which they actually attained some paternity; and (2) comparing the proportion of live embryos genetically assigned to parr of a given origin in those spawnings attended. Furthermore, the proportion of live embryos was used to compare efficiency at the individual level relative to parr phenotype, number of competing parr, order of attendance and absolute arrival time prior to spawning using Spearman rank order correlations.

RESULTS

Spawning characteristics and mating activities

We observed 61 spawning events in arena 1 and 58 in arena 2, and were able to precisely count the number of parr attending 58 of these in each arena. Parr identity to origin, based on visual observations, was ascertainable in 40 of 44 nests in arena 1, and 32 of 34 nests in arena 2. Five of the 30 nests analysed genetically did not have parr in attendance at the spawnings (confirmed by lack of parr paternity). Subsequent analyses thus focused on the 25 other nests. However, two of these were actually part of the same nest,

so their data were pooled, leaving a total of 24 nests (12 in each arena) to document parr reproductive success.

The total number of parr observed per spawning did not differ between the two arenas ($\chi^2 = 0.9$, d.f. = 1, $P = 0.39$). However, the occurrence per nest was significantly higher for farm parr (70% in arena 1 and 75% in arena 2) than both wild (47 and 34%, respectively) ($\chi^2 = 19.8$, d.f. = 3, $P < 0.001$) and hybrid parr (53 and 47%, respectively) ($\chi^2 = 8.8$, d.f. = 3, $P = 0.03$). There was no significant difference between wild and hybrid parr ($\chi^2 = 4.3$, d.f. = 3, $P = 0.24$). Moreover, the mean number of parr per spawning followed exactly the same pattern (farm vs. wild: $Z = 4.3$, $P < 0.001$; farm vs. hybrid: $Z = 2.9$, $P = 0.003$; hybrid vs. wild: $Z = 1.3$, $P = 0.21$; Fig. 1). There were no significant differences between arenas for parr of the same origin ($P > 0.05$).

Fertilization success

Three hundred and seventeen of the 1305 embryos (24.3%) analysed were fertilized by parr. Parr fertilization success within a nest was higher on average in arena 2 (mean: $33.8 \pm 39.2\%$, range: 0–100% in 12 nests) than in arena 1 (mean: $14.2 \pm 17.7\%$, range = 0–50.0% in 12 nests), but this difference was not significant (ANOVA using arcsine transformed data, $F_{1,22} = 1.7$, $P = 0.21$). Fertilization success was not a function of female identity, as there were no significant differences among females or among nests within a female (Kruskal–Wallis ANOVA: all $P > 0.05$). There was no difference between arenas in proportion of eggs fertilized by parr of the same origin ($Z = 0.4$, $P = 0.72$). However, farm parr had significantly higher fertilization success than wild parr ($Z = 2.6$, $P = 0.009$) and a similar, albeit marginally non-significant, trend was observed for hybrids ($Z = 1.9$, $P = 0.06$) (Table 1). The difference

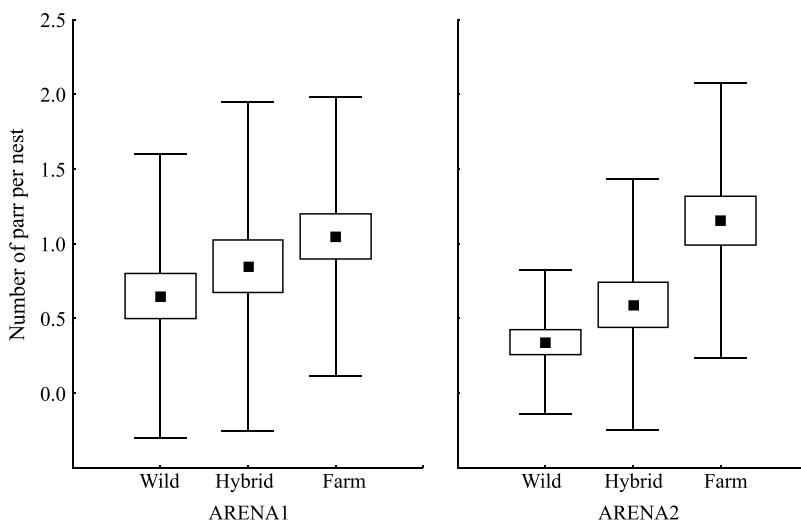


Figure 1 Box plots (mean/standard error/standard deviation) comparing number of parr per nest of differing origin in both arenas.

Table 1 Information on the fertilization success and efficiency of parr of differing origins for the 24 nests analysed genetically (12 in each breeding experiment). The data are mean \pm 1 standard deviation

	Number of parr with fertilized embryos		Fertilization success (percentage of eggs fertilized per nest analysed)		Fertilization efficiency (percentage of eggs fertilized per spawning attended)	
	Arena 1	Arena 2	Arena 1	Arena 2	Arena 1	Arena 2
Wild	2	1	3.6 \pm 12.0	5.7 \pm 19.7	5.4 \pm 14.7	17.1 \pm 34.2
Hybrid	6	2	4.5 \pm 7.5	11.2 \pm 26.5	7.8 \pm 8.6	22.4 \pm 35.3
Farm	4	6	6.1 \pm 12.1	16.9 \pm 27.7	9.1 \pm 14.1	25.4 \pm 31.0
Overall	12	9	4.7 \pm 10.5	11.3 \pm 24.6	7.4 \pm 12.4	22.6 \pm 31.3

between hybrid and farm parr was not significant ($Z = 1.0$, $P = 0.32$).

Fertilization efficiency

Group-level

In arena 1, wild parr obtained fertilization in two of the 10 spawnings (20%) they were present at compared with seven of nine (78%) for hybrids and eight of 12 (67%) for farm parr. In arena 2, wild parr were again the least successful, obtaining fertilization success in one out of four spawnings (25%) compared with four of seven (57%) for hybrids and nine of 11 (82%) for farm parr. There was no significant difference between arenas in fertilization efficiencies ($Z = 1.6$, $P = 0.12$). However, there were marginally non-significant differences, after Bonferonni sequential adjustments (initial level $\alpha = 0.017$), among parr origins with wild parr tending to be less successful than farm (Table 1, $Z = 2.2$, $P = 0.03$) and hybrid parr ($Z = 1.8$, $P = 0.07$), and the latter two not differing from each other ($Z = 0.6$, $P = 0.55$).

Individual-level

There was a significant difference in individual efficiency to fertilize eggs among arenas, with a higher efficiency in arena 2 than in arena 1 ($Z = 2.4$, $P = 0.02$). There was no difference in fertilization efficiency among individuals of different origin [Kruskal–Wallis: $H = 0.8$, $P = 0.67$]. The competitive environment had a significant effect on fertilization efficiency, with efficiency decreasing as the number of competing parr present at a given spawning increased (*Spearman* $r = -0.55$, $P = 0.01$) (Fig. 2). Across arenas, fertilization efficiency showed no significant relation with weight, length or condition factor (*Spearman* $r < 0.4$, $P > 0.05$). However, within arena 1, fertilization efficiency correlated positively with body weight ($r = 0.64$, $P = 0.03$), and non-significantly with length ($r = 0.51$, $P = 0.09$). In arena 2, there were no such trends. Across arenas, there was no relation between the order of arrival of a parr at a nest and its relative fertilization success (*Spearman* $r = -0.03$,

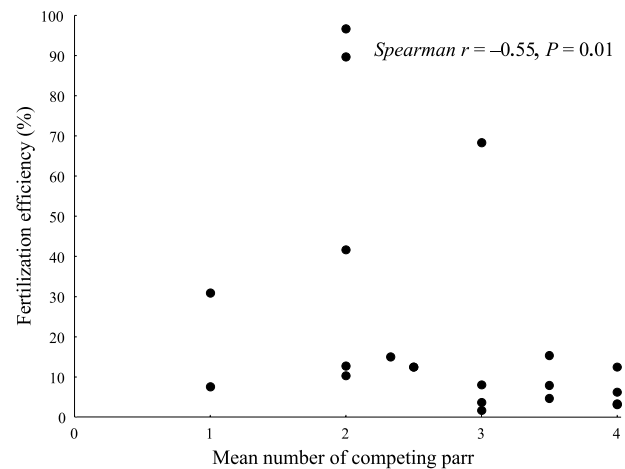


Figure 2 Relationship between mean number of competing parr at a nest (i.e. mean number of parr competing with the individual over all nests in which the individual obtained fertilization) and mean fertilization efficiency (i.e. mean proportion of live embryos within nests that an individual parr fertilized) for parr that obtained paternity based on genetic analysis.

$P = 0.89$). However, there was a marginally non-significant trend for relative fertilization success to increase with the arrival time at the nest prior to spawning (*Spearman* $r = 0.32$, $P = 0.1$).

Finally, we estimated the overall relative reproductive success of farm, hybrid and wild parr by multiplying the mean occurrence at spawning events by the mean fertilization efficiency. Farm parr had the highest relative reproductive success ($1.10 \times 17.23 = 18.91$), hybrids were intermediate ($0.74 \times 14.55 = 10.71$) and wild parr had the smallest relative reproductive success ($0.51 \times 9.31 = 4.78$). This means that hybrids had 56.6% and wild had 25.3% the success of farm parr.

Parr condition at the end of the experiment

ANCOVA revealed no significant effects of arena ($F_{1,44} = 2.1$, $P = 0.15$) or an arena-by-origin interaction ($F_{2,44} = 0.3$,

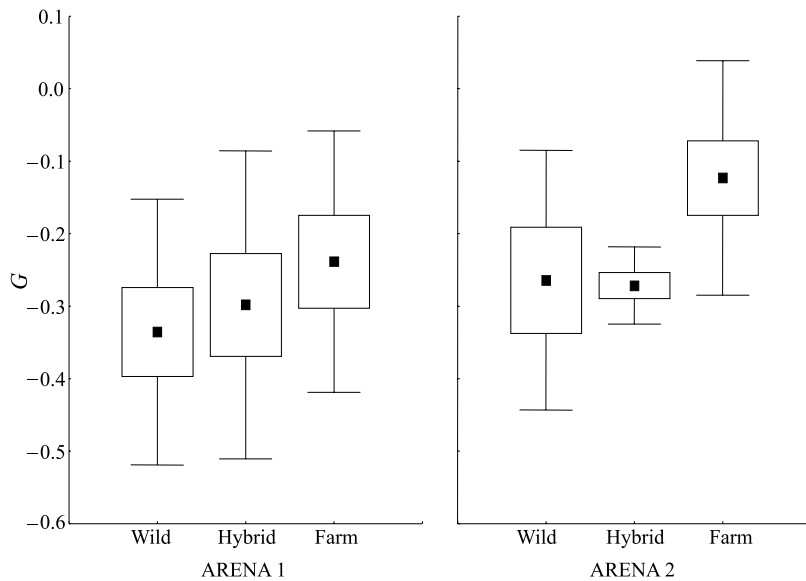


Figure 3 Box plots (mean/standard error/standard deviation) comparing growth (G) during the spawning period for parr of differing origin in both arenas. A negative sign on the Y-axis indicates a loss of weight during the experiment.

$P = 0.76$) on parr weight loss during the experiment. However, there was a marginally non-significant effect of origin ($F_{2,44} = 2.6$, $P = 0.09$), with farm parr tending to lose less weight than wild and hybrid parr (Fig. 3). There was no difference between arenas in number of parr incurring wounds during the experiment ($Z = 0.1$, $P = 0.90$). However, the occurrence of wounds was significantly lower in hybrid parr than in wild ($\chi^2 = 71.3$, $P < 0.001$) and farm parr ($\chi^2 = 61.4$, $P < 0.001$).

DISCUSSION

This study provides the first estimates of the fertilization success and efficiency of farm and hybrid Atlantic salmon parr. It also demonstrates that they have higher breeding and fertilization success than wild individuals reared in a common environment. Early maturing farm and hybrid males could thus be important vehicles promoting introgression of 'farm' genes into wild populations beyond the first, initial generation when sea-pen escapees invade. Such an effect ultimately threatens the long-term genetic integrity of native populations.

Origin of differences

As we compared farm fish with the wild population that principally founded the farm strain, and reared the groups under nearly identical environmental conditions, the differences in performance observed are more likely to be genetic in origin. While it is possible that the differences could have arisen due to maternal effects (e.g. egg size), this seems unlikely because such effects in salmonids are mainly

confined to periods immediately after emergence (e.g. Bagenal 1969; Einum & Fleming 2000a). Rather, the results support divergence due to domestication selection (i.e. evolutionary changes due to intentional and unintentional artificial selection) as the principal cause.

The superior access to, and higher fertilization efficiency at spawning of farm and, to a lesser extent, hybrid parr relative to wild parr may relate to differences in behaviour and physiology that are known to exist between the domesticated AquaGen fish and their principal wild founder population. In addition to improved freshwater growth (Fleming & Einum 1997), other fitness-related traits typical of the farm strain include increased aggression and decreased behavioural (Einum & Fleming 1997) and heart rate responses to simulated predation risk (Johnsson *et al.* 2001). These differences appear to relate to changes in growth endocrinology, particularly increased growth hormone (GH) expression (Fleming *et al.* 2002). Indeed, changes in the expression of these characters may translate into an increased willingness to take risks and be aggressive during breeding, and thus influence dominance, arrival time and other potential determinants of fertilization success.

Fleming *et al.* (1996, 2000) identified significant differences in spawning behaviour between anadromous farm (farm reared, farm origin) and wild (wild reared, wild origin) adults that resulted in reduced reproductive success for farm individuals. Such discrepancy with the present study likely involves both environmental and genetic effects. However, as we controlled for differences in rearing environment in the current study and subsequently observed farm fish to be successful, it suggests that the inferior breeding performance of the anadromous farm fish studied previously had a large

environmental component to its origin. Alternatively, it could be hypothesized that the genetic basis of reproductive behaviour differs between the two life-history stages, being only weakly correlated. This, however, would require further investigation.

Increased potential for introgression of farm genes

Previous studies have shown that not only do escaped, anadromous fish incur inferior breeding performance relative to wild fish, but that it is also sex-biased, with farm males incurring greater impairment than farm females (Fleming *et al.* 1996, 2000). Thus, much of the initial gene flow between wild and farm fish will more likely involve matings between wild males and anadromous farm females. The present findings, however, indicate that in subsequent generations this pattern is more likely to reverse, as some of the offspring of the initial invasion mature as male parr and breed. The superior breeding success of the farm and hybrid male parr relative to wild parr will thus enhance introgression in the second and potential subsequent generations.

To explore the potential contribution of age-1 parr possessing farm genes to the second generation, we used the estimates of absolute fertilization success for parr of different origins and adjusted them by the relative survival probabilities from the embryo to parr stage reported by Fleming *et al.* (2000) (farm juveniles had 70% the survival of wild and hybrid juveniles; see also McGinnity *et al.* 1997). This predicted that these parr could contribute as much as 12.0% ($[1 \times 11.5\% = \text{farm parr mean fertilization success} \times 0.70 \text{ relative survival}] + [1/2 \times 7.9\% = \text{hybrid mean fertilization success} \times 1.0 = \text{relative survival}]$) of the total male genes in the next year class of juveniles. Furthermore, our results showed that farm parr tended to lose less weight during the spawning period than other parr. This could potentially contribute to their survival, and enhance their probability of breeding in future seasons. Assuming there is little or no outbreeding depression in subsequent generations, this suggests that the previously estimated half-life of genetic differences (i.e. the rate at which the genetic difference between the donor and recipient population is halved) between wild and farmed salmon populations of about 10 generations (Fleming *et al.* 2000) may represent an upper limit. The presence of farm and/or hybrid parr on the spawning grounds of wild populations would most likely increase this rate of genetic introgression.

Determinants of individual success

The competitive environment that parr faced largely determined their ability to fertilize eggs. Indeed, we found a negative relationship between the number of parr competing with an individual and its efficiency to fertilize

eggs. This is congruent with findings of previous studies, where individual fertilization declines with the number of parr present at spawning (Hutchings & Myers 1988; Thomaz *et al.* 1997). It is also indicative of a density-dependent effect, which may generate negative frequency dependence between the alternative parr and anadromous male breeding tactics, and favour their maintenance (Gross 1996). This density-dependent relationship does not appear to be altered by the presence of parr of differing origins at spawning. We also showed that fertilization success tended to increase with time of arrival prior to spawning, with those parr being present in the nest for the longest period before oviposition having higher fertilization success. Proximity and/or priority in ejaculation has been shown to increase fertilization success among competing anadromous male salmon (Schroder 1982; Maekawa *et al.* 1994; Mjølnerød *et al.* 1998; but see Foote *et al.* 1997). Similarly, for mature male parr, early establishment at the nest may aid in obtaining and defending (e.g. prior resident effect) a privileged position for sneaking, increasing the chance fertilization success. Although, this may represent a trade-off against time to search and participate in other spawnings. There was, however, no significant relationship between order of nest entry and fertilization success. This may be explained by the observation that in almost every multi-parr spawning, participating parr were already present in the nest prior to oviposition. The effect of parr size (weight) on individual fertilization efficiency differed between arenas, being significant in arena 1 only. This may underline the fact that different traits might be favoured in different spawning contexts, and why some studies find an advantage of large parr size (Thomaz *et al.* 1997; Garant *et al.* 2002) and others do not (Jones & Hutchings 2001, 2002). For instance, in the present experiments, spawning peaked later and fewer anadromous males were involved in spawnings in arena 2 than in arena 1.

Fertilization vs. realized reproductive success

Fertilization success might not be representative of reproductive success in cases where survival is non-random in relation to fish origin. There was no evidence, however, that incubation survival was inferior for eggs fertilized by farm parr (results not shown). Yet, farm juveniles appear to incur higher mortality than wild juveniles post-hatching (McGinnity *et al.* 1997; Fleming *et al.* 2000). This juvenile mortality difference may partly relate to maternal effects, for example farm females produce smaller eggs than wild females (Fleming *et al.* 2000), and egg size is known to directly affect survival following emergence (Einum & Fleming 2000a, b). Thus the degree of differential survival would more likely be less for eggs parented by males than females of differing origins.

In the light of these considerations, we conclude that the higher estimates of reproductive success in farm and hybrid relative to wild parr documented in this study are realistic and conservative. Thus, early maturing farm and hybrid males may perpetuate significant gene flow in the second and subsequent generations of invasion by farm fish into native populations, hastening the threat to their genetic integrity.

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REFERENCES

- Armstrong, J.D., Einum, S., Fleming, I.A. & Rycroft, P. (2001). A method for tracking the behavior of mature and immature salmon parr around nests during spawning. *J. Fish Biol.*, 59, 1023–1032.
- Bagenal, T.B. (1969). Relationship between egg size and fry survival in brown trout, *Salmo trutta* L. *J. Fish Biol.*, 1, 349–353.
- Bohlin, T., Dellefors, C. & Faremo, U. (1990). Large or small at maturity—theories on the choice of alternative male strategies in anadromous salmonids. *Ann. Zool. Fennici*, 27, 139–147.
- Clifford, S.L., McGinnity, P. & Ferguson, A. (1998). Genetic changes in Atlantic salmon (*Salmo salar*) populations of Northwest Irish rivers resulting from escapes of adult farm salmon. *Can. J. Fish. Aquat. Sci.*, 55, 358–363.
- Crozier, W.W. (1993). Evidence of genetic interaction between escaped farmed salmon and wild Atlantic salmon (*Salmo salar* L.) in a Northern Irish river. *Aquaculture*, 113, 19–29.
- DeWoody, J.A., DeWoody, Y.D., Fiumera, A.C. & Avise, J.C. (2000). On the number of reproductives contributing to a half-sib progeny array. *Genet. Res. Camb.*, 75, 95–105.
- Einum, S. & Fleming, I.A. (1997). Genetic divergence and interactions in the wild among native, farmed and hybrids Atlantic salmon. *J. Fish Biol.*, 50, 634–651.
- Einum, S. & Fleming, I.A. (2000a). Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). *Evolution*, 54, 628–639.
- Einum, S. & Fleming, I.A. (2000b). Highly fecund mothers sacrifice offspring survival to maximize fitness. *Nature*, 405, 565–567.
- Fiske, P., Lund, R.A., Østborg, G.M. & Fløystad, L. (2001). Escapes of reared salmon in coastal and riverine fisheries in the period 1989–2000. *NINA Oppdragsmelding*, 704, 1–26 (in Norwegian with an English abstract).
- Fleming, I.A. (1995). Reproductive success and the genetic threat of cultured fish to wild populations. In: *Protection of Aquatic Biodiversity. Proceedings of the World Fisheries Congress, Theme 3* (eds). Phillip, D.P., Epifanio, J.M., Marsden, J.E. & Claussen, J.E. Oxford and IBH publishing Co., New Delhi, pp. 117–135.
- Fleming, I.A. (1996). Reproductive strategies of Atlantic salmon: ecology and evolution. *Rev. Fish Biol. Fish.*, 6, 379–416.
- Fleming, I.A., Jonsson, B., Gross, M.R. & Lamberg, A. (1996). An experimental study of the reproductive behaviour and success of farmed and wild Atlantic salmon (*Salmo salar*). *J. Appl. Ecol.*, 33, 893–905.
- Fleming, I.A. & Einum, S. (1997). Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication. *ICES J. Mar. Sci.*, 54, 1051–1063.
- Fleming, I.A., Hindar, K., Mjølnerød, I.B., Jonsson, B., Balstad, T. & Lamberg, A. (2000). Lifetime success and interactions of farm salmon invading a native population. *Proc. R. Soc. Lond. B*, 267, 1517–1523.
- Fleming, I.A., Agustsson, T., Finstad, B., Johnsson, J.I. & Björnsson, B.T. (2002). Effects of domestication on growth physiology and endocrinology of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.*, 59, 1323–1330.
- Foote, C.J., Brown, C.S. & Wood, C.C. (1997). Spawning success of males using alternative mating tactics in sockeye salmon, *Oncorhynchus nerka*. *Can. J. Fish. Aquat. Sci.*, 54, 1785–1795.
- Garant, D., Dodson, J.J. & Bernatchez, L. (2000). Ecological determinants and temporal stability of within-river population structure in Atlantic salmon (*Salmo salar* L.). *Mol. Ecol.*, 9, 615–628.
- Garant, D., Fontaine, P.-M., Good, S.P., Dodson, J.J. & Bernatchez, L. (2002). The influence of male parental identity on growth and survival of offspring in Atlantic salmon (*Salmo salar*). *Evol. Ecol. Res.*, 4, 537–549.
- Gjedrem, T., Gjøen, H.M. & Gjerde, B. (1991). Genetic origin of Norwegian farmed Atlantic salmon. *Aquaculture*, 98, 41–50.
- Gross, M.R. (1996). Alternative reproductive strategies and tactics: diversity within sexes. *TREE*, 11, 92–98.
- Gross, M.R. (1998). One species with two biologies: Atlantic salmon (*Salmo salar*) in the wild and in aquaculture. *Can. J. Fish. Aquat. Sci.*, 55 (Suppl. 1), 131–144.
- Gross, M.R., & Repka, J. (1998). Stability with inheritance in the conditional strategy. *J. Theor. Biol.*, 11, 92–98.
- Hansen, L.P., Håstein, T., Nævdal, G., Saunders, R.L., & Thorpe, J.E. (editors) (1991). Interactions between cultured and wild Atlantic salmon. *Aquaculture*, 98, 1–324.
- Hindar, K., Ryman, N. & Utter, F. (1991). Genetic effects of cultured fish on natural populations. *Can. J. Fish. Aquat. Sci.*, 48, 945–957.
- Hutchings, J.A. & Jones, M.E.B. (1998). Life-history variation and growth rate thresholds for maturity in Atlantic salmon. *Salmo salar*. *Can. J. Fish. Aquat. Sci.*, 55 (Suppl. 1), 22–47.
- Hutchings, J.A. & Myers, R.A. (1988). Mating success of alternative maturation phenotypes in male Atlantic salmon, *Salmo salar*. *Oecologia (Berlin)*, 75, 169–174.

- Hutchings, J.A. & Myers, R.A. (1994). The evolution of alternative mating strategies in variable environments. *Evol. Ecol.*, 8, 256–268.
- IUCN. (1997). Conserving vitality and diversity. In: *Proceedings of the World Conservation Congress Workshop on Alien Invasive Species* (eds Rubec, C.B.A. & Lee, G.O.). Published in partnership with IUCN species survival commission and the North American wetlands conservation council (Canada), 96 p.
- Johansson, J.I., Höjesjö, J. & Fleming, I.A. (2001). Behavioural and heart rate responses to predation risk in wild and domesticated Atlantic salmon. *Can. J. Fish. Aquat. Sci.*, 58, 788–794.
- Jones, M.W. & Hutchings, J.A. (2001). Influence of male parr body size and mate competition on fertilization success and effective population size in Atlantic salmon. *Heredity*, 86, 675–684.
- Jones, M.W. & Hutchings, J.A. (2002). Individual variation in Atlantic salmon fertilization success: implications for effective population size. *Ecol. Appl.*, 12, 184–193.
- Jordan, W.C. & Youngson, A.F. (1992). The use of genetic marking to assess the reproductive success of mature male Atlantic salmon parr (*Salmo salar* L.) under natural spawning conditions. *J. Fish Biol.*, 41, 613–618.
- Kellogg, K.A. (1999). Salmon on the edge. *TREE*, 14, 45–46.
- Maekawa, K., Nakano, S. & Yamamoto, S. (1994). Spawning behaviour and size-assortative mating of Japanese charr in an artificial lake-inlet stream system. *Env. Biol. Fish.*, 39, 109–117.
- McGinnity, P., Stone, C., Taggart, J.B., Cooke, D., Cotter, D., Hynes, R. *et al.* (1997). Genetic impact of escaped Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. *ICES J. Mar. Sci.*, 54, 998–1008.
- Mjølnerød, I.B., Refseth, U.H., Karlsen, E., Balstad, T., Jakobsen, K.S. & Hindar, K. (1997). Genetic differences between two wild and one farmed population of Atlantic salmon (*Salmo salar*) revealed by three classes of genetic markers. *Hereditas*, 127, 239–248.
- Mjølnerød, I.B., Fleming, I.A., Refseth, U.H. & Hindar, K. (1998). Mate and sperm competition during multiple-male spawning of Atlantic salmon. *Can. J. Zool.*, 76, 70–75.
- O'Reilly, P.T., Hamilton, L.C., McConnell, S.K. & Wright, J.M. (1996). Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Can. J. Fish. Aquat. Sci.*, 53, 2292–2298.
- Rice, W.R. (1989). Analysing tables of statistical tests. *Evolution*, 43, 223–225.
- Schroder, S.L. (1982). The influence of intrasexual competition the distribution of chum salmon in an experimental stream. In: *Salmon and Trout Migratory Behavior Symposium* (eds Brannon, E.L. & Salo, E.O.). School of Fisheries, Univ. Washington, Seattle, pp. 275–285.
- Slettan, A., Olsaker, I. & Lie, Ø. (1995). Atlantic salmon, *Salmo salar*, microsatellites at the *SSOSL25*, *SSOSL85*, *SSOSL311*, *SSOSLA17* loci. *Anim. Genet.*, 26, 281–282.
- StatSoft. (1999). *STATISTICA for Windows [Computer program manual]*. StatSoft Inc., Tulsa, OK, USA.
- Taggart, J.B., McLaren, I.S., Hay, D.W., Webb, J.H. & Youngson, A.F. (2001). Spawning success in Atlantic salmon (*Salmo salar* L.): a long-term DNA profiling-based study conducted in a natural stream. *Mol. Ecol.*, 10, 1047–1060.
- Taylor, E.B. (1991). A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. *Aquaculture*, 98, 185–207.
- Thomaz, D., Beall, E. & Burke, T. (1997). Alternative reproductive tactics in Atlantic salmon: factors affecting mature parr success. *Proc. R. Soc. Lond. B*, 264, 219–226.
- Webb, J.H., Hay, D.W., Cunningham, P.D. & Youngson, A.F. (1991). The spawning behavior of escaped farmed and wild Atlantic salmon (*Salmo salar* L.) in a northern Scottish river. *Aquaculture*, 98, 97–110.
- Youngson, A.F., Martin, S.A.M., Jordan, W.C. & Verspoor, E. (1991). Genetic protein variation in Atlantic salmon in Scotland: comparison of wild and farmed fish. *Aquaculture*, 98, 231–242.
- Youngson, A.F., Webb, J.H., MacLean, J.C. & Whyte, B.M. (1997). Frequency of occurrence of reared Atlantic salmon in Scottish salmon fisheries. *ICES J. Mar. Sci.*, 54, 1216–1220.
- Youngson, A.F., Dosdat, A., Saroglia, M. & Jordan, W.C. (2001). Genetic interactions between marine finfish species in European aquaculture and wild conspecifics. *J. Appl. Ichthyol.*, 17, 153–162.

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