

Genetic impact on two wild brown trout (*Salmo trutta*) populations after release of non-indigenous hatchery spawners

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Abstract: A genetically marked hatchery strain of brown trout (*Salmo trutta*) was employed to study the genetic impact from non-indigenous hatchery fish on wild stocks. The hatchery spawners were released in autumn 1989 into the spawning localities of two wild trout stocks in River Øyreselv, Norway. The F₁ generation was sampled and genotyped at the 0+, 1+, and 2+ stages. Juveniles carrying the genetic markers were found in both localities, proving that the introduced spawners had spawned among themselves and with the wild stocks. The genetic contribution from the hatchery fish was estimated at 19.2 and 16.3% at the 0+ stage in the two wild stocks. Estimates of survival rates of 0+ trout revealed that survival was nearly three times higher in wild trout than in hybrids of wild and introduced trout, possibly because of a difference between introduced and wild stocks in size of eggs and alevins. The frequency of the marker alleles in the F₁ generation declined during the 2-year observation period.

Résumé : Nous avons utilisé une lignée d'élevage génétiquement marquée de truite brune (*Salmo trutta*) pour étudier l'impact génétique des poissons d'élevage non indigènes sur les stocks sauvages. À l'automne 1989, nous avons relâché des géniteurs élevés en pisciculture dans les frayères de deux stocks de truite sauvages de la rivière Øyreselv, en Norvège. Nous avons prélevé un échantillon de la génération F₁ et en avons établi les génotypes aux stades 0+, 1+ et 2+. Nous avons retrouvé dans les deux localités des juvéniles portant les marqueurs génétiques, ce qui prouve que les géniteurs introduits se sont croisés entre eux et avec les stocks sauvages. Les contributions génétiques des sujets d'élevage du stade 0+ des deux localités ont été estimées à 19,2 et à 16,3%. Chez les truites du stade 0+, le taux estimatif de survie était trois fois plus élevé pour les sujets sauvages que pour les hybrides entre sujets sauvage et introduit, sans doute à cause d'une différence entre les stocks sauvages et introduits quant à la taille des oeufs et des alevins. La fréquence des allèles marqueurs chez la génération F₁ a diminué au cours des deux années d'observation.

[Traduit par la Rédaction]

Introduction

To what extent do fish that escape or are released from hatcheries and fish farms affect the genetic characteristics and ecology of wild populations? This is an important question in the discussion of possible impacts of escaped fish on wild stocks in Norway as well as in many other countries. In Norway, the discussion has intensified as the production of farmed Atlantic salmon (*Salmo salar*) has expanded, with a corresponding increase in the number of escaped farmed salmon in coastal areas and rivers. At the same time the number of harvested wild salmon in many rivers has been showing a downward trend. The production of farmed salmon in Norway alone has increased from 4 000 t in 1980 to about 260 000 t in 1995, while the annual yield of wild salmon has varied between 1000 and 2500 t during the last 30 years according to Norwegian fishery statistics. The number of wild salmon spawners in Norwegian rivers has been estimated at 100 000 individuals (Ståhl and Hindar 1988), while the official estimate of the

number of escaped salmon in 1989 was 2 million. The number of hatchery smolts produced exceeds 90 million, while the number of wild salmon smolts leaving Norwegian rivers has been estimated at approximately 6 million. Thus, the number of cultivated salmon is high compared with the number of wild salmon. However, the genetic impact is not dependent on numerical relationships alone. The degree of impact also depends on the extent of genetic adaptation of wild populations, the degree of genetic differentiation between wild and cultivated populations, and on the performance of escaped cultivated individuals (Fleming and Gross 1993).

There is little empirical data on genetic adaptation at the population level and on genetic impact by various categories of cultivated populations on wild stocks of salmonids. For this reason, discussions of genetic impact from aquaculture, ocean ranching, and enhancement programmes often rely on theoretical considerations. Genetic marking has been recommended as a valuable tool in studies on reproductive success of released and escaped fish and their impact on wild stocks (Allendorf and Utter 1979; Shaklee 1983; Taggart and Ferguson 1984; 1986; Skaala and Jørstad 1994).

The purpose of our experiment was to study the short term impact by non-indigenous hatchery brown trout (*Salmo trutta*) on genetic characteristics and population dynamics of indigenous stocks in a natural habitat.

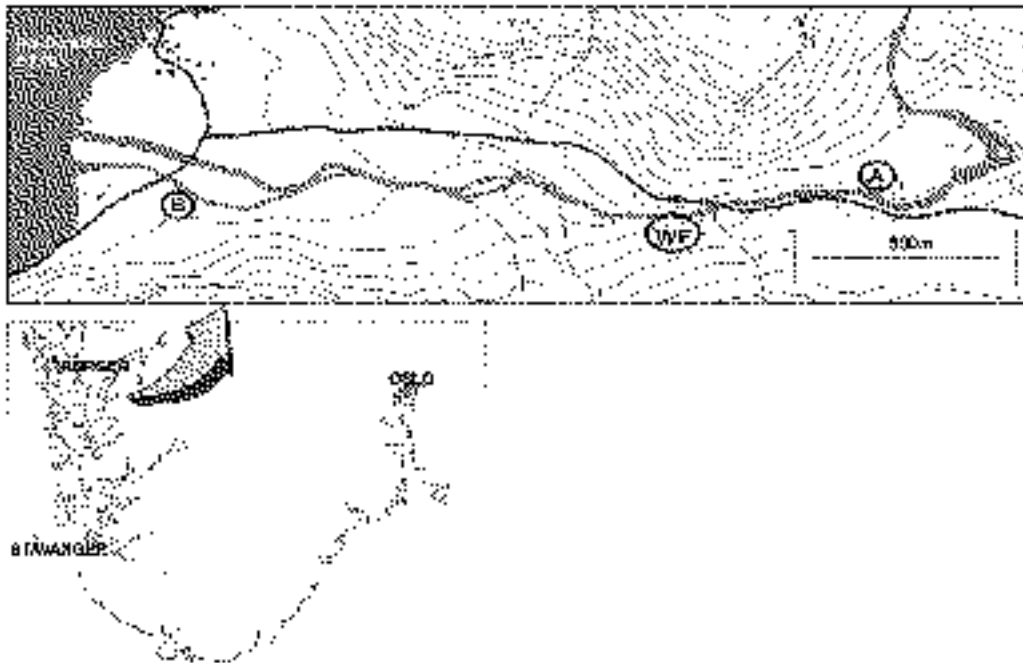
The brown trout was chosen as a model species in this experiment for several reasons. Firstly, it is hypothesized

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Fig. 1. Map of southern Norway with the field locality indicated: enlarged map of River Øyreselv showing above (A) and below (B) waterfall (WF) sections.



that adverse effects of population mixing would be most pronounced and most readily detected in species where a large portion of the total genetic variability is divided among populations. The brown trout is one of the most polymorphic vertebrate species known, with reproductive barriers occurring between populations separated by small distances, and with a wide variation in life-history characters (Ryman 1983; Ferguson 1989). Secondly, suitable genetic markers are abundant in brown trout as opposed to Atlantic salmon. Finally, as brown trout are smaller than Atlantic salmon, and inhabit smaller rivers and creeks, there are also practical advantages for experimental design in using this species as a model.

Materials and methods

The area

The River Øyreselv drains into the Hardangerfjord on the west coast of Norway (Fig. 1). The river has a population of Atlantic salmon, but anadromous and resident brown trout is the dominant species by numbers. A waterfall impassable to anadromous fish is located about 1.5 km from the fjord. The lower part of the river is divided in two parallel branches, each 375 m long. Two sections of the river were chosen for the experiment; section A above the waterfall, and section B, the smallest side branch below the waterfall, where the discharge is generally less than $1\text{--}2\text{ m}^3\cdot\text{s}^{-1}$. Higher discharges usually occur during snowmelt and during late autumn. Resident and anadromous brown trout spawn in section A and B, respectively. In section A, a large part of the area is suitable for spawning, while in section B the river bed is dominated by large stones and boulders, with small areas of suitable spawning substrate. The average width of the river channel in section B is $10.0 \pm 2.9\text{ m}$ (mean \pm SD). During our sampling, the temperature ranged from $0\text{--}2.5^\circ\text{C}$ in winter to $8.5\text{--}12.5^\circ\text{C}$ in autumn.

Genetically marked non-indigenous hatchery spawners were released in both sections in autumn 1989. The released spawners

were all first-generation hatchery fish grown under standard rearing conditions in fibreglass tanks and fed only commercial dry feed. Thus, they had no previous experience with natural river environments, and limited physical conditioning compared with wild spawners. In section A, video observations of the spawning behaviour of hatchery and wild trout were made (Skaala et al. 1993). In section B, a mark-recapture study on juveniles was carried out to estimate the number and survival rate of offspring resulting from wild and introduced trout, and from combinations of these, and to assess changes in population parameters due to the potential genetic impact. Thus, the major effort was concentrated in section B.

Genetic markers and electrophoresis

In 1986, production of a genetically marked trout strain was initiated by selecting and mating individuals with specific phenotypes (Skaala and Jørstad 1988). During 1988, genetic information on a number of wild trout populations was recorded to find a suitable locality for the experiment, and electrophoretic studies were conducted. These included the following 12 enzyme systems: aspartate aminotransferase (AAT, E.C. 2.6.1.1), alcohol dehydrogenase (ADH, E.C. 1.1.1.1), adenylate kinase (AK, E.C. 2.7.4.3), creatine kinase (CK, E.C. 2.7.3.2), esterase (EST, E.C. 3.1.1.-), glycerol-3-phosphate dehydrogenase (G3PDH, E.C. 1.1.1.8), isocitrate dehydrogenase (IDHP, E.C. 1.1.1.42), lactate dehydrogenase (LDH, E.C. 1.1.1.27), malate dehydrogenase (MDH, E.C. 1.1.1.37), malic enzyme (MEP, E.C. 1.1.1.40), glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9), and phosphoglucosmutase (PGM, E.C. 5.4.2.2), putatively encoded by 32 loci. Allelic frequencies were calculated according to Ferguson (1980). Details of the electrophoretic methods are given by Skaala and Jørstad (1987). Two types of genetic markers were used in each section, a visible marker (*PIGM**) and an isozyme marker (Table 1). The visible marker includes a genetic polymorphism with an allele (f) causing a fine spotting pattern on the body and fins that can be easily distinguished from the large spots commonly found in brown trout (Skaala and Jørstad 1987, 1988). Juveniles homozygous for this variant allele lack parr marks. Heterozygous individuals are intermediate between the fine-spotted

Table 1. Allele frequencies in the marker loci (*LDH-5**, *MDH-2**, and *PIGM**) of the introduced fine-spotted trout strain, of the wild trout in sections A and B in River Øyreselv, and in neighbouring wild populations.

Population	<i>N</i>	<i>LDH-5*</i> 100	<i>MDH-2*</i> 152	<i>PIGM*</i> (<i>f</i>)
Hatchery	50	0.730	0.800	1.000
River Øyre A	73	0.760	0.021	0.000
River Øyre B	103	0.034	0.272	0.000
River Enes	73	0.036	0.224	0.000
River Fosså	71	0.014	0.120	0.000
Lake Opsanger	83	0.059	0.524	0.000
Lake Gygrastol	96	0.000	0.240	0.000

and common genotypes in appearance, and juveniles have irregular and disrupted parr marks (Fig. 2). The two isozyme markers used were *MDH-2*152* (section A) and *LDH-5*100* (Section B). Samples of the 1990 year-class were collected in 1990 (0+), 1991 (1+), and 1992 (2+) to record genotypic distributions and calculate allelic frequencies. The genetic contribution made by the introduced stock was estimated by means of allelic frequency proportionality (Taggart and Ferguson 1986) as:

$$\left(\frac{P_n - P_m}{P_n - P_f} \right) \times 100$$

where P_n is the allelic frequency in the native population, P_m in the mixed population, and P_f is the allelic frequency in the introduced hatchery population. Genotypic distributions in marker loci were tested for heterogeneity by using a *G*-test (Sokal and Rohlf 1969), and differences in allelic frequencies were tested by the BIOSYS-1 PC program package (Swofford and Selander 1981).

Abundance of trout

To address the potential ecological implications of induced gene flow, in terms of changes in production (Crozier 1993), population dynamic studies were incorporated in the experiment in section B. The number of wild spawners in section A was estimated by diving surveys and fish counting, and a sample of adult fish was collected by gillnetting to record the lengths and weights of spawners. The 0+ parr were sampled by divers and by electrofishing. In section B the number of spawners was recorded by electrofishing during the spawning seasons. Based on population estimates and genetic marking, the numbers in each year-class and the survival of offspring resulting from wild and reared fish were assessed. In section B, the number of 0+, 1+, and older parr were estimated by a mark-recapture method ((Chapman's adjusted Peterson method) (Ricker 1975) in August 1989, March and August 1990, February and August 1991, March and September 1992, and February 1993. Confidence limits for the estimates were obtained from Ricker (1975, appendix II), using the number of recaptures of marked fish as the entering variable.

The fish were captured by an electric shocker, anaesthetized with benzocaine, measured (total length in millimetres) and marked either with alcian blue dye at the base of the pelvic, ventral, anal, and caudal fins, using a Jet inoculator (Hart and Pitcher 1969; Pitcher and Kennedy 1977) or by removing the adipose fin. All samplings, except in August 1990, were carried out at low and approximately similar stream flows, in which the whole stream bed could be waded. In August 1990, the first sampling was carried out during a flood, and had to be restricted to a narrow zone along the

banks of the stream, resulting in a low capture probability for 1+ and older parr.

In August 1989, the 0+ parr were not marked. Thus, no Petersen estimate was obtained. Likewise, no Peterson estimates of 0+ parr were obtained in March 1990 and February 1991 because of the low number of recaptures. For these dates, the number of 0+ was estimated by catch per unit effort (CPUE), according to the equation $C = qN$, where C is the number caught during the first sampling run, q is the catchability, and N is the number of parr present. For the other months, estimates of numbers of 0+ were obtained both by mark-recapture and CPUE. The catchability of 0+ parr was estimated according to the relationship $q = aL^b$, where L is the mean length in millimeters and a and b are constants. The length-classes used in these calculations were obtained from Borgstrøm and Skaala (1993), and q for each cohort of 0+ was estimated separately for each sampling month (Table 2). Survival (S) estimates of 0+ from August–September to February–March have been obtained by using the estimated numbers from the CPUE method ($S = N_1/N_2$, where N_1 and N_2 are numbers of parr in August–September and February–March, respectively) and by the use of marked fish, as described by Ricker (1975) ($S = R_{12} M_2/M_1 (R_{22} + 1)$, where M_1 and M_2 are number of marked fish in August–September and February–March, respectively, and R_{12} and R_{22} are the numbers of recaptures of the two markings after the second marking).

Release of non-indigenous hatchery spawners

In autumn 1989, spawners homozygous for the fine-spot allele were released in spawning areas of wild brown trout in River Øyre. The numbers of reared and genetically marked trout released in section A were 56 (43 males and 13 females), and 104 (67 males and 37 females) in section B. The individual length of the released spawners varied between 25.5 and 42.5 cm in section A, and between 32.0 and 42.5 cm in section B, corresponding to masses between 0.2 and 1.0 kg. Each spawner was tagged individually with a Floy anchor tag. Diving surveys and video recordings were made during late autumn and winter to estimate the number of released trout still present at the spawning grounds.

Results

Genetic impact

All samples of wild trout from year-classes hatched before the release experiment matched the expected Hardy–Weinberg distribution. The visible marker (fine-spotted) was not found in wild trout in the River Øyreselv or in the nearby rivers. The *MDH-2*152* allele common in the marked strain ($p = 0.800$) was rare in the population in section A ($p = 0.02$). The *LDH-5*100* allele, present at a high frequency ($p = 0.730$) in the marked strain, was present at a low frequency ($p = 0.03$) in the anadromous brown trout in section B in River Øyreselv as well as in the neighbouring rivers (Table 1). Thus, two independent genetic markers were available in each section, one visible and one allozyme.

In forty-three 0+ parr sampled in section A above the falls in October 1990, two individuals homozygous for the visible marker, and seven heterozygous individuals were identified, giving a frequency of 0.128 for this allele (Table 3). Genotypic distributions in the 1990 year-class and previous year-classes differed in both the visible marker ($p < 0.0001$) and the *MDH-2** marker ($p < 0.01$). In contrast to the earlier year-classes, the 1990 year-class deviated significantly from the expected Hardy–Weinberg distribution in the pigmentation-controlling locus as well as in *MDH-2**. In 1992, homozygotes for the *MDH-2** marker allele were not detected in a

Fig. 2. Juvenile phenotypes from previous test matings corresponding to those found in the 1990 year-class in the River Øyreselv. Fine-spotted homozygotes, nos. 1 and 3 (from above); common, nos. 2 and 4; and heterozygotes, nos. 5, 6 and 7.



Table 2. Parameter values a and b in the model $q = aL^b$, the corresponding q values of 0+ parr and the average length of 0+ parr.

Date	a	b	r	q	Length (cm)*
Aug. 25, 1988	0.4031×10^{-3}	1.3763	0.9796	0.1129	6.0 ± 0.7
Aug. 30, 1989	0.2875×10^{-5}	2.3586	0.9921	0.0335	5.2 ± 0.6
Mar. 28, 1990	0.1789×10^{-3}	1.5058	0.9771	0.0788	5.8 ± 0.8
Aug. 29, 1990	—	—	—	—	—
Feb. 27, 1991	0.1524×10^{-3}	1.5951	0.9727	0.0807	5.2 ± 0.7
Aug. 28, 1991	0.9014×10^{-2}	0.7435	0.9391	0.1701	5.4 ± 0.7
Mar. 5, 1992	0.1624×10^{-2}	1.0934	0.9391	0.1203	5.6 ± 0.9
Sept. 2, 1992	0.1570×10^{-2}	1.1206	0.8225	0.1258	5.1 ± 0.6
Feb. 18, 1993	0.6013×10^{-3}	1.2484	0.9714	0.0936	5.7 ± 0.6

*Values are means \pm SDs.

Table 3. Genotype and allele frequencies for the marker loci (*MDH-2** and *PIGM**) in section A, River Øyreselv, in the introduced strain and in different year-classes of the local wild stock.

Sample and year-class	N	<i>MDH-2*</i>			p 152	<i>PIGM*</i>			p f
		100 100	100 152	152 152		WW	WF	FF	
Hatchery 1987	50	1	18	31	0.800	0			
Wild 1985–1986	73	70	3	0	0.021	73	0	0	0.000
Wild 1988–1989	33	31	2	0	0.029	33	0	0	0.000
Wild 1990 (0+)	43	32	7	4	0.174	34	7	2	0.128
Wild 1990 (2+)	93	73	20	0	0.108	—	—	—	—

Note: The 1990 year-class was sampled in 1990 and 1992 as 0+ and 2+, respectively. WW, homozygous wild individuals; WF, heterozygotes; FF, homozygous non-indigenous individuals.

sample of 93 individuals, including 20 heterozygotes. Thus, the frequency of this allele had dropped to 0.108.

In section B, 0+ parr from the 1990 year-class carrying the genetic markers were detected in samples from 1990, 1991, and 1992 (Table 4). However, in this section the visible marker was only found in heterozygous form. In October 1990, 20 heterozygotes were recorded in this year-class, giving a frequency of the marker allele of 0.075, higher ($p < 0.0001$) than the frequency of this allele, (0) before the experiment. The frequency of the fine-spotted allele dropped significantly from 0.075 to 0.030 between October 1990 and February 1991. By June 1991 the frequency was 0.027.

The frequency of the *LDH-5** marker in the indigenous trout stock in section B varied from 0.027 to 0.034 in year-classes between 1986 and 1989, while in the 1990 year-class, this allele was found in 26 of 134 individuals, giving a frequency of 0.149, which is higher ($p < 0.0001$) than in previous year-classes. By June 1991, the frequency of the *LDH-5** marker had decreased to 0.106. Deviations observed from expected Hardy–Weinberg equilibrium were significant in both the pigmentation controlling locus and in *LDH-5**. In the 1992 sample, when the 1990 year-class had reached the 2+ stage, the frequency had dropped further to 0.092. Genotypic distributions differed between the 1990 year-class and the previous year-classes in both the visible marker ($p < 0.0001$) and in the *LDH-5** marker ($p < 0.0001$).

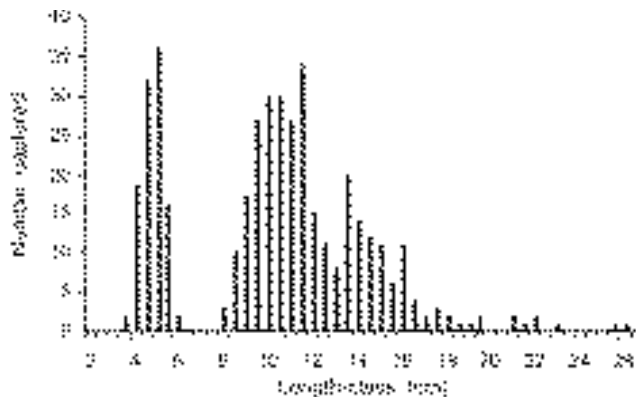
In section A, there was good agreement between the change in allelic frequencies estimated by the visible marker and the isozyme marker. The observed numerical change between the 1988–1989 year-classes and the 1990 year-class was 0.128 for the visible marker, and 0.144 for the isozyme marker (Table 3). In section B, the numerical change in allelic frequencies were 0.075 in the visible marker and 0.122 in the isozyme marker (Table 4). The change observed in the isozyme marker was, therefore, comparable with the change observed in section A, while the change observed in the visible marker was somewhat lower.

The genetic contribution of the introduced hatchery trout in section A by September 1990 was 19.2% based on the allozyme marker, *MDH-2**, and 12.8% based on the visible marker. In section B, the genetic contribution made by the introduced trout by September 1990, was 16.3% based on the allozyme marker *LDH-5**, and 7.5% based on the visible marker. By June 1991, the genetic contribution from introduced trout in section B had dropped to 10.9% according to the allozyme marker *LDH-5*100*, and to 2.7% according to the visible marker (Table 4).

Abundance of trout

The number of wild spawners in section A was estimated at approximately 100. In section B, the number of wild anadromous spawners with lengths between 25 and 65 cm, registered

Fig. 3. Length distribution of juvenile trout in section B, River Øyreselv.



during electrofishing, has been below 12 each autumn. Assuming a catchability of $q = 0.5$, the total number of wild spawners has been below 24, with the number of female spawners not exceeding 10–15. The individual lengths of the introduced spawners lay within the length range of wild spawners.

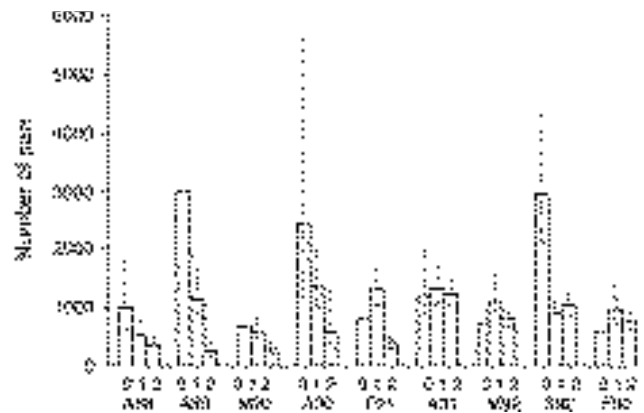
The anadromous trout in section B in River Øyre generally smolt at 3 years of age, at a length of 12–14 cm. Thus, few larger and older parr were present, and three modes can be identified in the length distributions (Fig. 3) corresponding to the 0+, 1+, and 2+ juveniles.

For single cohorts, the Petersen method and the CPUE method gave similar estimates of 0+ in section B (Fig. 4, Table 5). However, the abundance of 0+ parr shows considerable annual variation. The annual autumn numbers of 0+ varied from 800 to 3000, while the same cohorts in February–March had lower abundances and smaller annual variations in numbers, (400–700), indicating a high mortality rate from August–September to February–March in all years (Table 5).

From August 1990 to February 1991 the frequency of heterozygous 0+ decreased from 0.15 to 0.06, which gives a much lower survival rate of heterozygous 0+ compared with the indigenous 0+ cohort (Table 5). In the same period, the estimated survival rate of indigenous 0+, based on marked fish, was nearly identical to the survival rate based on the estimated number of fish (Table 5), indicating insignificant immigration of indigenous 0+ during the period August 1990 – February 1991. Thus, the lower survival of heterozygotes compared with indigenous 0+ is not explained by immigration of indigenous 0+. However, in August–September, the 1+ parr were more abundant than 0+ of the same cohort in the previous period (Fig. 4). From this it follows that immigration from the main river may occur from midwinter to the next autumn. Thus, due to the higher mortality rate of heterozygous 0+, and later on also due to immigration of indigenous trout, the frequency of introduced genetic material became increasingly lower.

The mean length of the 0+ parr in August 1988 was 6.0 ± 0.7 cm; ($n = 122$) compared with 5.2 ± 0.6 cm; ($n = 88$) in August 1989, and 4.5 ± 0.9 cm; ($n = 143$) in August 1990 (Table 2). In October 1990 the mean lengths of wild 0+ and of heterozygous 0+ resulting from crosses between wild and introduced trout were 4.7 ± 0.7 cm and 4.0 ± 0.6 cm, respectively, while in March 1991, there was no difference in size

Fig. 4. Number of 0+, 1+ & 2+ trout at the various sampling dates in section B, River Øyreselv. Vertical bars give SD for the Petersen estimates.



(5.1 ± 0.6 cm and 5.1 ± 0.3 cm, respectively) between the two groups.

Discussion

Genetic impact

There was a considerable contribution of genetic material from the introduced hatchery trout in the F_1 generation, calculated at the 0+ stage, in both of the wild populations in the river. Thus, we demonstrated that not only did the non-indigenous hatchery trout engage in spawning activity, but also genetic material was actually incorporated in the gene pools of the wild anadromous and freshwater resident stocks. However, taking into account the relatively large number of spawners of non-indigenous origin compared with the number of wild spawners (about 4:1 in favour of non-indigenous spawners), the genetic contribution from non-indigenous population was less than expected (19.2 and 16.3% in sections A and B, respectively).

According to the population estimates and the genetic markers, we recorded a decline in the frequency and absolute number of individuals carrying non-indigenous alleles in the F_1 generation over about 2 years. The results suggest that the higher mortality rate observed in hybrid offspring may be caused by smaller size of individuals and lower capability to obtain and defend territories. The territorial behaviour of trout parr may result in a downstream drift of nonterritorial fish, which eventually die (Elliott 1986, 1987). Elliott (1989) found a critical survival time after fry emergence, and fry without territories decreased from a high number before the critical time to almost none afterwards. Although we have no data on the number of hatched fry and their mortality rate after hatching until August, the number of fry probably decreased considerably from spring to August. If the difference in survival rate from August to February was established already from the fry stage, the genetically marked fry may actually have been more abundant at time of hatching than offspring from wild fish. Thus, the declining frequency of the introduced genetic material in the F_1 generation may be due to a higher mortality rate of the heterozygotes compared to the native 0+. Immigration of parr to section B from the main river, above the branching

Table 4. Genotype and allele frequencies for the marker loci (*LDH-5** and *PIGM**) in section B, River Øyreselv, in the introduced strain and in different year-classes of the local wild stock. The 1990 year-class was sampled in 1990, 1991, and 1992 at the 0+, 1+, and 2+ stage, respectively.

Sample and year-class	<i>LDH-5*</i>					<i>PIGM*</i>			
	<i>N</i>	<i>90</i> <i>90</i>	<i>90</i> <i>100</i>	<i>100</i> <i>100</i>	<i>p</i> <i>100</i>	<i>WW</i>	<i>WF</i>	<i>FF</i>	<i>p</i> <i>f</i>
Hatchery 1987	50	3	21	26	0.730	0	0	50	1.000
Wild 1985–1988	102	95	7	0	0.034	73	0	0	0.000
Wild 1988–1989	55	52	3	0	0.027	55	0	0	0.000
Wild 1990 (0+)	134	101	26	7	0.149	114	20	0	0.075
Wild 1990 (1+)	—	—	—	—	—	126	8	0	0.030
Wild 1990 (1+)	66	53	12	1	0.106	102	6	0	0.027
Wild 1990 (2+)	60	49	11	0	0.092	—	—	—	—

Note: The 1990 year-class was sampled in 1990 and 1992 as 0+ and 2+ stage, respectively. *WW*, homozygous wild individuals; *WF*, heterozygotes; *FF*, homozygous non-indigenous individuals.

Table 5. Number and survival rates of indigenous (W) and hybrid (H) 0+ parr in section B.

Date	<i>N</i>	<i>N</i>	<i>S_m</i>	<i>S_n</i>	<i>S_n</i>
	W	H	W	W	H
Aug. 30, 1989	3150	0	—	0.208	—
Mar. 28, 1990	655	0	—	—	—
Aug. 29, 1990	2060	364	0.371	0.351	0.130
Feb. 27, 1991	723	46	—	—	—
Aug. 28, 1991	823	0	0.292	0.514	—
Mar. 5, 1992	423	0	—	—	—
Sept. 2, 1992	2289	0	0.261	0.257	—
Feb. 18, 1993	566	0	—	—	—

Note: Population numbers were obtained by the CPUE method (Borgstrøm and Skaala 1993). *S_n*, survival rate based on estimated number of 0+; *S_m*, survival rate based on recaptures of marked indigenous 0+ trout.

point, may further gradually reduce the frequency of heterozygotes from the 1+ stage. Therefore the genetic contribution in F_1 from the non-indigenous spawners may be regarded as a minimum estimate.

The genetically marked strain was originally derived from a population in a mountain lake at 1243 m above sea level. It has been documented that brown trout from lakes at high altitudes have smaller eggs than trout living at lower altitudes (Sømme 1941; Ø. Skaala, unpublished data). According to the length distribution of wild and heterozygous parr, there is a shift in the length distribution towards larger sizes between October 1990 and February 1991. The heterozygous 0+ parr were smaller in October than the wild 0+ parr, while their lengths were almost identical in February. Because the winter temperature in the river is low, it is unlikely that growth in length alone can explain the observed increase of about 1 cm

in the mean length from October 1990 to February 1991 in 0+ parr. This may reflect size-specific mortality, i.e., higher mortality of the heterozygotes.

Genetic marking is a well established method that can be used to study gene flow (Gharrett and Seeb 1990). The genetic markers in the introduced hatchery trout stock enabled the gene flow from introduced to indigenous stocks to be recorded and quantified. In general, the visible marker was less effective than the isozyme markers in discriminating between alleles originating from introduced and wild stocks. The benefit of the visible marker, however, was that it permits nondestructive sampling and recording. An explanation for the difference between the visible and the biochemical genetic marker in section B, may be that heterozygotes for the visible marker are more easily misclassified than homozygotes are. Because of the lack of homozygotes in section B, this will be more important in section B than in section A. Therefore, the genetic contribution from introduced hatchery spawners in section B may be higher than indicated by the visible marker in this section. A few other experiments to estimate the reproductive success of reared fish have been conducted (Seeb et al. 1986; Skaala et al. 1990, and references therein; Crozier 1993; Moran et al. 1994; Crozier and Moffett 1995). Chilcote et al. (1986) used genetic marking to compare the reproductive success of transplanted, nonlocal hatchery steelhead trout (*Oncorhynchus mykiss*) with that of wild steelhead during the subyearling and smolt stages. They found that naturally spawning hatchery trout contributed substantially to the recruitment of wild juveniles. However, the reproductive success of their hatchery trout was considerably lower than that of the wild counterparts, as was also found in our study. Leider et al. (1990), who continued the work of Chilcote et al. (1986), found that the reproductive success of hatchery trout continued to decrease and was only 0.108–0.129 measured at the adult stage of the offspring.

Implications for management of aquaculture and wild stocks

The introduced spawners in our study were all first-generation hatchery fish without any experience with natural river

environments, and limited physical conditioning compared with wild spawners. Nevertheless, the hatchery fish participated in spawning activities in both sections of the river and spawned among themselves as well as with individuals of the indigenous anadromous and freshwater resident trout stocks. The present experiment does not give any data on differences in spawning success or reproductive success between sexes. However, it is known from other studies on hatchery salmonids (Fleming and Gross 1993) that females have higher success than males, who have a very low success. In section B, there are indications that fry heterozygous for the marker loci are smaller than homozygous wild type, which is to be expected if mothers were of hatchery origin.

Although significant genetic changes were recorded in both of the indigenous trout populations, the number of genetically marked 0+ parr present in the river the year after spawning was much lower than expected from the number of genetically marked, introduced spawners, demonstrating a low spawning success of the introduced hatchery fish. Reduced performance and a low reproductive success of hatchery individuals in natural habitats may be caused by: (1) natural stock differences, (i.e., genetic differences between the stocks acquired through previous differentiation in natural environments), (2) genetic changes in the hatchery stock arising in the artificial environment, and (3) nongenetic factors, such as physiological stress or lack of stamina. In our study, the difference observed may be related to the pronounced genetic differences between the hatchery population and the wild stocks, differences in spawning behaviour and competitive ability of spawners, differences between reared and wild populations in the size of eggs and larvae, or behavioural differences of fry. As suitable spawning substrate in section B is limited, the released fish may have been forced to spawn in suboptimal habitats, with a higher risk of egg losses. It has been reported that escaped Atlantic salmon concentrate their spawning in the lower parts of rivers, which also may be a result of competition with wild spawners (Webb et al. 1991).

It is likely that the acclimatization and reproductive success of hatchery fish in natural environments depends on several circumstances, such as the life-stage at which the fish escape. For example, fish that escape at an early stage in life may suffer high mortality, but survivors have more time to acclimatize before spawning; therefore, survivors may reproduce more successfully than fish that escape as ripe spawners just before the spawning season.

Changes in productivity have been mentioned as one potential implication of introduction of non-indigenous genetic material (Crozier 1993). In our experiment there was a massive introduction of non-indigenous hatchery spawners in the spawning habitat just before the beginning of spawning in the wild population. Still, the presence of a high number of introduced spawners seems to have had little influence on the reproductive success of the wild anadromous brown trout, as the number of 0+ parr originating from wild parents was actually higher in August 1990 compared with the numbers in the two preceding years. Nevertheless, we have no information on the performance of the F_1 generation beyond the 2+ stage or on the F_2 generation.

Although the relative size of wild and hatchery eggs in our study deviates from the common situation, it serves to focus attention on a potentially important competitive point.

Farmed salmonids are selectively bred for increased growth rate and size at maturity, which may imply that escaped spawners will be large compared with their wild counterpart. Thus, the size of eggs from farmed females will tend to be larger than eggs from wild females as there is a positive correlation between individual fish length and egg diameter (L'Abée-Lund and Hindar 1990). Given that this assumption is valid, fry hatched from farmed eggs will be larger than wild fry and thus have a competitive advantage at the early stage even though they may be less adapted at later stages. In conclusion then, hatchery fish may compete successfully at the spawning grounds and mate among themselves as well as with wild individuals. If a sufficiently high number of offspring in the F_1 generation compete successfully and survive, the outcome may be a change in the genetic characteristics of the wild populations. On the other hand, if their offspring compete less successfully, with a correspondingly high mortality in F_1 , a whole year-class may be greatly reduced.

It is generally known that hybridization between populations can produce a wide range of effects from heterosis to outbreeding depression. There is still little empirical information on the magnitude of induced gene flow between wild and cultivated salmonid populations and on the biological consequences of it in terms of short-term or long-term changes in productivity (Waples 1991). From the present data we conclude that a single introduction of hatchery fish is not necessarily critical to the indigenous population, as the spawning success of hatchery fish may be low, and the survival rate of their offspring may be reduced due to a poor adaptation. However, when more frequent inputs of hatchery fish occur, as may often be the situation, the genetic characteristics of the wild stocks will be altered, even when the hatchery fish has a lower reproductive success. If the F_1 hybrids display increased fitness due to heterosis, the impact will be even greater.

Natural and induced fluctuations in population abundances involve complex mechanisms, such as changes in competition within and among cohorts, and in predation between age- and size-classes. It is highly probable that escapement and release of cultivated organisms in large numbers will affect wild populations ecologically and genetically, also through these mechanisms, in particular when the non-indigenous organisms breed successfully and produce unusually high densities of individuals in a year-class. If a numerous year-class is dominated by offspring from a non-indigenous stock, this year-class may prey heavily at least on the two consecutive year-classes. Thus, a numerous year-class with a high frequency of offspring from a non-indigenous source may lead to one or more weak year-classes and a wild population that is even more vulnerable to invading individuals.

Because the number of species and the biomass of cultivated organisms are rapidly increasing, it is likely that escapement and release of cultivated organisms is going to raise many more questions in the near future, in particular when taking into account development and potential commercial use of transgenic organisms. In the public as well as in the scientific community, there has been and will be public and scientific discussion about the usefulness and importance of preserving wild stocks. Many of the important

questions within this discussion can only be answered through a combination of theoretical and empirical studies on stock discreteness and genetic adaptation in wild populations. For most species, such studies have only barely begun.

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