

Assessment of three strategies practiced by fishery managers for restoring native brown trout (*Salmo trutta*) populations in Northern French Alpine Streams

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Accepted for publication September 10, 2010

Abstract – The efficiency of three strategies carried out by fisheries managers to restore native Mediterranean brown trout populations threatened by non-native Atlantic populations were assessed. The strategies tested were (i) genetic refuge area where stocking is banned, (ii) stocking with Mediterranean fry and (iii) translocation of wild Mediterranean spawners. Using two discriminatory microsatellite loci between Atlantic and Mediterranean alleles, we compared the genetic composition of samples before and after the changes of practices. The three strategies had several detectable effects in the standing populations causing strong temporal changes in departures from Hardy–Weinberg equilibrium and linkage equilibrium between loci and the apparition of new Mediterranean alleles. The significant reductions in the proportions of Atlantic alleles observed over time can mostly be explained by the disappearing of the pure non-native Atlantic trout after the stopping of hatchery releases. The results, however, also suggest that the active strategies carried out by managers led to intraspecific introgression between both non-native Atlantic and native Mediterranean strains.

Key words: genetic refuge; native brown trout; restoration strategies; stocking; translocation

Introduction

During the last two decades years, the conservation of native populations of salmonid fishes, declining because of overharvesting, habitat degradation or invasions by non-native fish, has become an important world-wide challenge for researchers and wildlife managers (Waples & Hendry 2008). The brown trout (*Salmo trutta* L.) displays a high genetic and phenotypic (i.e., morphology, behaviour, life-history) diversity across its native range in Europe (Elliott 1994; Bernatchez 2001; Klemetsen et al. 2003). There is evidence that the intraspecific diversity of brown trout reflects adaptations to local environment (Hindar et al. 1991; Ryman 1991; Pakkasmaa & Piironen 2001; Reisenbichler et al. 2003). Thus, in terms of

conservation, maintaining the genetic integrity of local brown trout populations as much as possible is crucial to ensure the evolutionary processes (Taylor 1991; Ryman et al. 1995; Laikre et al. 1999; Stockwell et al. 2003; Utter 2004).

In France, population genetics studies of brown trout populations revealed the co-occurrence of two genetically distinct brown trout forms, i.e., Atlantic and Mediterranean trout named after their assumed origins according to the hydrographic drainage (Guyomard 1989; Bernatchez et al. 1992; Estoup et al. 2000; Launey et al. 2003). In the French North Alpine hydrographic catchment area belonging to the Mediterranean Basin, the Mediterranean populations of brown trout are considered as the native form (Guyomard 1989; Largiadèr et al. 1996; Launey et al.

2003). Until recently, this area has been stocked with hatchery stocks of Atlantic origin for more than one century (Krieg & Guyomard 1985; Launey et al. 2003).

Several studies (Barbat-Leterrier et al. 1989; Beaudou et al. 1994; Poteaux et al. 1998) have shown that the Atlantic trout has replaced Mediterranean populations originally present in many streams of the Mediterranean basin. In contrast, in some Mediterranean streams, despite similar stocking practices, native populations showing little genetic introgression by the Atlantic gene pool (Barbat-Leterrier et al. 1989; Largiadèr & Scholl 1996; Launey et al. 2003) are still present.

In the Dranse River system, a typical mountain stream network, the spatial distribution of both native Mediterranean and non-native Atlantic trout are as follows: the median parts of the system harbour nearly pure Mediterranean populations, whereas Atlantic populations issued from stocking inhabit the isolated upper parts of the rivers (Caudron 2008). The conservation of these remnant native populations displaying low introgression rates should be integrated in the management programmes to protect the well-adapted local populations. This may be particularly important for populations inhabiting mountain streams characterised by unstable and difficult environmental conditions. For instance, Champigneulle et al. (2003), describing the spawning habitat of a native Mediterranean brown trout population in a Dranse tributary, showed that the spawning sites used by native fish were less destroyed by floods than the classical riffle habitat used by non-native trout. The authors suggested that this diversification of spawning sites may represent an important adaptive factor for the native population to persist in hydrologically unstable mountain streams. Then, fishery managers of this area were gradually carried out different conservation and restoration measures. Three main strategies have been carried out at different locations of this large system: (i) genetic refuge area where stocking is banned, (ii) stocking with Mediterranean fry sectors inhabited by Atlantic populations and (iii) translocation of wild Mediterranean spawners in Atlantic populations. These strategies are most promoted ones restoration programmes of native salmonids populations (Maitland 1995; Moritz 1999; Crivelli et al. 2000; Schmetterling 2003; Carofino et al. 2008; Araguas et al. 2009). A detailed assessment and monitoring of such programmes will be crucial for an early detection of unintended negative effects on the populations and to refine future restoration programmes.

Here, we report a temporal genetic analysis before and after the change of practices for three main restoration strategies carried out by managers to detect their potential effects on the wild populations in three different sites, to attempt an assessment of the efficiency of these restoration strategies.

Materials and methods

Location and characteristics of the study area

The study was carried out on the system of the Dranse River located in the northern French Alps, which is the second largest affluent of Lake Geneva and belongs to the Mediterranean catchment (Fig. 1). The rivers of this system are typical mountain streams of the alpine zone with in particular a fragmented habitat because of the presence of many natural or artificial impassable obstacles. In this catchment area, the access of migratory lake-dwelling trout from Lake Geneva to the river is restricted to a 14 km long stretch of the main river because of the presence of impassable obstacles. Thus, all upstream parts of the Dranse system are inhabited by stream-dwelling brown trout.

Several genetic studies (Guyomard 1989; Largiadèr et al. 1996) showed the presence of native Mediterranean populations of brown trout in the median part of the Dranses River. These Mediterranean populations are considered as being native because all of the stocking operations have been performed with hatchery stocks of Atlantic origin (Krieg & Guyomard 1985; Launey et al. 2003). Both forms also show distinct differences in morphology and in body pigmentation. Several studies indicate that the phenotypic characters as the body-shape, the number of parr marks, the colour of the anal and dorsal fins and the spotting patterns may be used for a rough visual distinction between Atlantic and Mediterranean trout (Lascaux 1996; Mezzera et al. 1997; Aparicio et al. 2005; Monet et al. 2005).

The synthesis of the genetic results obtained in this area (Guyomard 1989; Largiadèr et al. 1996; Launey et al. 2003; Caudron 2008) indicated that the populations of both Mediterranean and Atlantic trout showed a particular geographical distribution (Fig. 1). In the median part of the system, nearly pure populations of Mediterranean origin were found, whereas Atlantic populations isolated by obstacles were identified in the upper parts of this hydrographic network (Fig. 1). There is some evidence that these upstream Atlantic populations were originally introduced by stocking: (i) the stocking history indicates that intensive stocking had taken place in these upstream parts of the Dranses system and (ii) genetic analyses indicated that there was no detectible genetic difference between these upstream Atlantic populations, and the hatchery strains that were used for stocking these rivers (Estoup et al. 2000).

Restoration strategies practiced by managers

Since the end of the nineteenth century, this area has been stocked with brown trout from hatchery stocks of

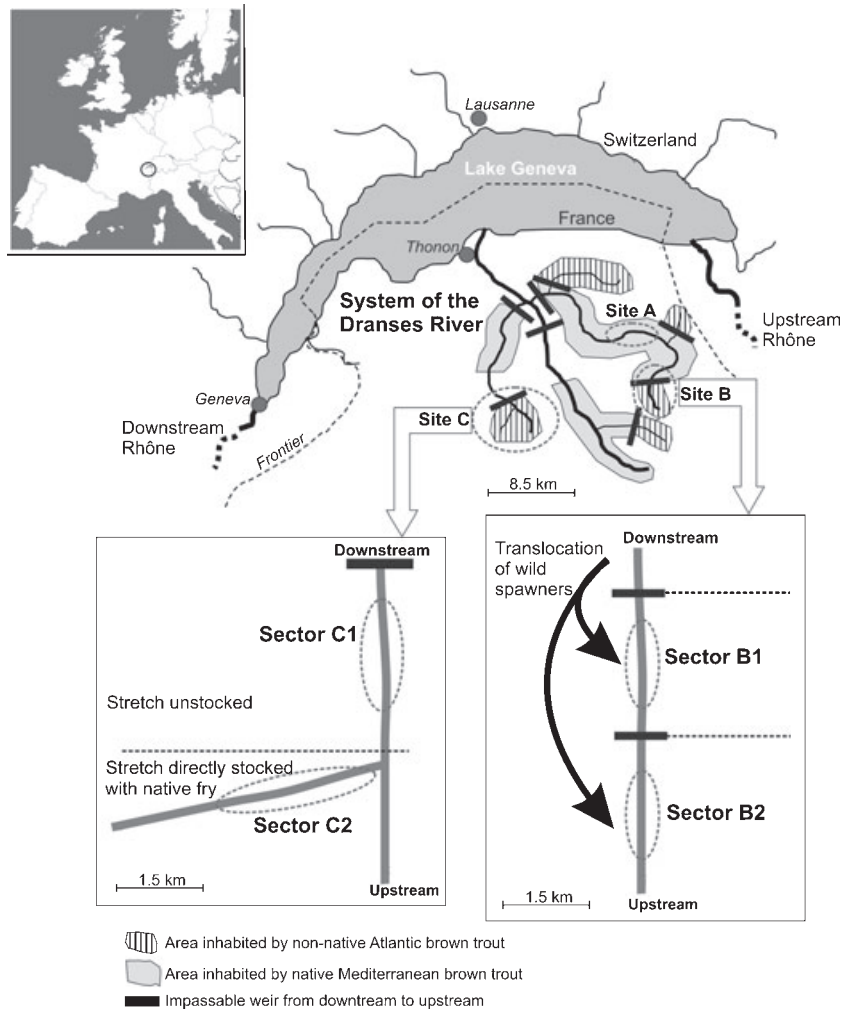


Fig. 1. Location and characteristics of the different sites and sectors studied in the Dranses River System.

Atlantic origin. The different Atlantic hatchery stocks used by fishery managers showed a high genetic similarity among them and a high genetic differentiation with the brown trout populations of the French Mediterranean catchment area (Krieg & Guyomard 1985; Beaudou et al. 1994; Presa et al. 1994; Largiadèr et al. 1996; Berrebi et al. 2000; Launey et al. 2003). During the second half of the twentieth century, between 700,000 and 1,000,000 of Atlantic trout have been stocked per year at fry stage (4–6 cm in length). Fifteen years ago, in view of the pattern of repartition in the Dranse system between both native Mediterranean and non-native Atlantic trout, fishery managers decided to change their traditional practices and to carry out different strategies of restoration in the aim to establish Mediterranean populations of native trout in upstream where Atlantic trout issued from stocking were present. The three main strategies carried out by managers were as follows: (i) stopping the stocking with Atlantic hatchery trout often called genetic refuge strategy (Araguas et al. 2008, 2009), (ii) stopping the stocking with Atlantic hatchery trout

and translocation of wild spawners issued from a native population and (iii) stocking with fry produced from native Mediterranean spawners.

Previous studies and field sampling

The implementation *in situ* of these three restoration strategies by fishery managers and the existence of genetic data or samples in this area resulting of the many studies carried out in the past provided an unique opportunity to assess the genetic effect of these strategies on the wild populations. Thus, a genetic monitoring of the brown trout populations has been carried out in three different sites (A, B and C) where genetic data or samples were available before the change of managing practices (Fig. 1). In the study area, the rivers were only inhabited by stream-dwelling trout as because of impassable obstacles the area cannot be accessed by lake-dwelling spawners from Lake Geneva.

At site A, the genetic refuge strategy (stopping the stocking with Atlantic hatchery trout) has been carried

out since 1996. This population has been sampled in 1995, before the stocking with Atlantic trout was stopped, and after, every year between 1996 and 2000 and also in 2006. This sampling scheme allowed to monitor the population in 2000 and 2006, respectively, after 5 and 11 years of a genetic refuge strategy and to follow the cohorts 1996 and 1997, which were the two-first generations after the stocking was stopped.

At site B, the situation was as follows: the genetic refuge strategy has been carried out during 4 years between 1996 and 1999, then in autumn 1999 and 2000, respectively, 83 and 140 wild spawners have been caught from downstream of the impassable weir in a relatively pure native Mediterranean population and translocated to upstream in two isolated sectors inhabited by Atlantic trout (Fig. 1). The translocated wild spawners had not been genetically analysed before the translocations. However, these fish had been caught in a native population showing a frequency of Atlantic alleles of only 8% (Caudron 2008). Furthermore, only individuals showing typical phenotypic characters of Mediterranean trout (Lascaux 1996; Mezzera et al. 1997; Aparicio et al. 2005; Monet et al. 2005) had been selected for the translocation.

All the translocated spawners have been marked by cutting the adipose fin to distinguish them of the wild population during the sampling operations realised after the translocation. The population has been sampled in two sectors (B1 and B2) during three different periods: in 1996–1997 before the change of practice, in 1999–2000 after 4 years of genetic refuge and in 2003 to follow the population issued from the translocated spawners.

At site C, the stocking strategy using Mediterranean fry produced from recent captive breeding stock founded with native wild spawners has been realised between 1999 and 2004. The captive Mediterranean breeding stock has been founded with 98 families derived from local wild spawners caught in the Dranse d'Abondance River showing Mediterranean genotypes at three diagnostic markers, Str541, Str591 and Str791 (Caudron et al. 2006). Samples fry stocked in 2000 and 2003 were genotyped (Appendix S1), to check for unintended losses of genetic variability in the progeny issued from the breeding stock. Between 1999 and 2004, the release of the Mediterranean fry at fed fry stage (2–3 cm in length) in the study river area had been carried out in May, when hydrological conditions were favourable. Each year, between 5600 and 7300 individuals were released in this area resulting in varying yearly stocking density of 0.5–0.7 fry per m².

This population has been monitored in two sectors C1 and C2. The sector C2 is located upstream in a stretch of river directly stocked with Mediterranean fry, whereas the sector C1 is located in a stretch of river not directly stocked but located immediately

downstream of the area stocked (Fig. 1). In this site, the samples have been realised in 1999 influenced by the stocking with Atlantic trout, and in 2004 and 2006, respectively, after 4 and 6 years of stocking with Mediterranean fry.

Concerning the fragmentation level of the habitat in the study area with regard to fish movement, several impassable obstacles prevented the movement of trout between the site C and the two others sites studied. Furthermore, upstream migration between sites A and B and between sectors B1 and B2 was impossible but not downstream movements. Within site C, the downstream and upstream migrations among the C1 and C2 sectors were possible. At all site, the samples of trout were collected continuously in autumn by electrofishing over a long stretch of river to be representative of the populations present at the different sites. At the site B2, the survey by electrofishing did not allow to catch any trout during the 1999–2000 period after 3 years of genetic refuge strategy. For each trout sampled, some scales and a fin clip were taken. The fin clips were stored in 95% ethanol for genetic analysis. The age of each trout was determined by scalimetry to assign trout to its cohort. At site A, the samples allowed to monitor the population at each age-class between 1+ and 4+ years. At sites B and C, only the 2+- and 3+- year-old individuals were analysed to obtain samples with comparable age structure in the samples of all time periods that were compared. The sampling periods, the different restoration strategies and the number of trout analysed per sampling site and cohort are summarised in Table 1.

Table 1. Samples analysed in each site and sector investigated for the three restoration strategies monitored.

Year/Periods	Management Situations	Samples				
	SITE A	1+	2+	3+	4+	Total
1995	Before Genetic Refuge	14	16	16	5	51
2000	5 years of Genetic Refuge	17	49	49	17	132
2006	11 years of Genetic Refuge	20	16	23	13	72
Cohort 1994	Before Genetic Refuge	14	46	20	26	106
Cohort 1996	First generation after Genetic Refuge	38	52	29	17	136
Cohort 1997	Second generation after Genetic Refuge	46	37	49	–	132
	SITE B (Age 2+/3+)	Sector B1		Sector B2		Total
1996–1997	Before Genetic Refuge	82		22		104
1999–2000	3 years of Genetic Refuge	73		No fish		73
2003	After Translocation	94		34		128
	SITE C (Age 2+/3+)	Sector C1		Sector C2		Total
1999	Stocking with ATL	38		24		62
2004	4 years of stocking with MED	33		36		71
2006	6 years of stocking with MED	33		35		70

Genotyping

Samples were genotyped at two microsatellite markers, Str541 and Str591, which are located on two distinct linkage groups (BT2 and BT7, Gharbi et al. 2006). These microsatellite loci have been shown to be diagnostic between Atlantic domesticated stocks and unstocked Mediterranean trout populations over a large part of the Mediterranean area including France, Italy, Greece and Spain (Estoup et al. 2000; Launey et al. 2003; R. Guyomard, unpublished data). This diagnostic attribute and the ability of these two markers to trace back each allele to its geographical origin (Atlantic or Mediterranean) has been validated through the genotyping at two microsatellites loci (Str542 and Str592, respectively), which are physically closely linked to Str541 and Str591, respectively. The rationale of this approach has been detailed in Estoup et al. (1999, 2000).

DNA was extracted from the tissues with magnetic beads (MagneSil BLUE; Promega, Madison, WI, USA). PCR amplifications were performed with the Multiplex PCR Kit (Qiagen, Hilden, Germany) on a GeneAmp PCR system 9700 (ABI, Foster City, CA, USA). PCR was carried out in 10 μ l reaction volume containing 5 μ l Multiplex PCR Master Mix, 3 μ l dH₂O, 1 μ l DNA and 1 μ l primer mix (2 pmol· μ l⁻¹ each primer). Cycling conditions consisted of an initial denaturation of 15 min at 95 °C followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 90 s and sequence extension at 72 °C for 1 min and a final extension step at 72 °C for 10 min. A quantity of 1 μ l of the PCR product was added to a buffer containing a LIZ 600 size standard ladder (ABI), and two microsatellite loci were analysed on an automated DNA sequencer (ABI PRISM 3130 XL, Foster City, CA, USA). Genotypes were determined with the Genemapper 3.0 (ABI) software and checked by eye.

Statistical analyses

Allele frequencies, average number of alleles, observed and expected heterozygosities were computed with GENETIX 4.05 (Belkhir et al. 1996–2004). Deviations from Hardy–Weinberg equilibrium (HWE) were tested using the exact probability test of Guo & Thompson (1992) available in GENEPOP 3.4 (Raymond & Rousset 1995). Genotypic linkage disequilibrium between the two loci was tested by Fisher's exact tests using GENEPOP 3.4. These tests used a Markov chain method (1000 dememorisation steps, 100 batches and 1000 iterations per batch). To increase the power of the analysis, tests were also made after pooling the Atlantic and Mediterranean alleles in two categories for each sample (cf. Mezzera & Largiadèr 2001).

For the pooled allele data, we also calculated standardised linkage disequilibrium coefficients D' (Lewontin 1964) based on gametic linkage disequilibrium coefficients estimated with the Hill's (1974) maximum likelihood method using the software MATHEMATICA version 7.0 (Wolfram Research).

The percentage of the Atlantic alleles was estimated as the average frequencies of Atlantic alleles at the Str541 and the Str591 locus (i.e., number of Atlantic alleles at Str541 and Str591 found in a sample divided by the total number of allele observed in this sample). The 95% confidence intervals of Atlantic allele frequencies were calculated based on Beyer's (1986) tables (values based on binomial distribution). Statistical differences in proportions of Atlantic alleles between the different periods for each restoration strategy monitored were tested with a chi-square test. Each individual was given a hybrid index score between 0 and 4, according to the observed number of hatchery specific alleles at the two diagnostic loci. Thus, a hybrid index score of 4 would be assigned to a pure hatchery trout, whereas hybrid index score of 0 would be assigned to a pure native.

Results

With the exception of site A, several additional Mediterranean alleles were observed at all sites, after a change in the management practices.

In the 2003 sample of the sector B1, two additional Mediterranean alleles, 166 and 194 with frequencies of 3% and 10%, respectively, were found at locus Str591. These two alleles have been observed in the sample of the wild spawners translocated in 1999 and 2000 with frequencies, respectively, of 6% and 28%. In the 2003 sample of the sector B2, four additional Mediterranean alleles (166, 190, 194, 200), observed also in the sample of the wild spawners translocated, were found at Str591 (see Appendix S1).

Genetic analysis revealed four and eight additional Mediterranean alleles at Str591, respectively, in the sector C1 and C2, in 2006, after the stocking with Mediterranean fry (see Appendix S1).

Site A

The multilocus tests performed with all the alleles and with alleles pooled into two categories (Mediterranean vs. Atlantic) reveal significant departures from HWE only for the 1995 sample ($P < 0.01$). This apparently resulted from the occurrence of seven pure Atlantic individuals (on a sample of 51), which exhibited Atlantic alleles only at both loci, as the test was no longer significant ($P > 0.05$) when these seven individuals were discarded. The 2000 and 2006 samples did not deviate significantly from the HWE

($P > 0.05$). The tests showed significant linkage disequilibrium for both 1995 and 2000 samples, whereas for the 2006 sample, the two loci were in linkage equilibrium. Congruently, the standardised coefficients indicate a decay of the linkage disequilibrium in 2006 sample when compared to 1995 and 2000 samples (Table 2).

The percentage of Atlantic alleles decreased significantly between 1995 and 2000 ($P < 0.01$) and remained similar between 2000 and 2006. The distributions of the hybrid index score were significantly different between 1995 and both 2000 and 2006 samples ($P < 0.05$) and were similar between 2000 and 2006. Indeed, the percentage of pure hatchery trout decreases from 12% to 1.5% between 1995 and 2000 (Fig. 2).

For the 1995 sample, the level of Atlantic alleles was significantly higher in the 1+ and 2+ age-classes than in 3+ ($P < 0.01$) and 4+ age-classes ($P < 0.05$). For the 2000 sample, the rates of the Atlantic alleles were similar between the three age-classes 1+, 2+ and 3+ and were significantly lower in the 4+ age than in the 2+ and 3+ ages ($P < 0.05$). For the 2006 sample, the percentage of Atlantic alleles did not differ between age and classes ($P > 0.05$) (Fig. 3).

The percentage of Atlantic alleles decreased over time between each age-class only for the cohort 1994 influenced by stocking with trout of Atlantic origin

(Fig. 3). The differences were only significant (chi-square test, $P < 0.05$) between the 1+ and 4+ age-classes. For the 1996 and 1997 cohorts, the levels of Atlantic alleles remained similar between age and classes.

For the three monitored cohorts, the distribution of the hybrid index score did not differ significantly (chi-square test, $P > 0.05$) between age and classes (Fig. 3).

Site B

At sector B1, the multilocus tests performed with all the alleles and with alleles pooled into two genotype categories did not yield any significant departures from HWE ($P > 0.05$) for both time periods 1996–1997 and 1999–2000. Significant linkage disequilibrium was observed for the 1996–1997 period, whereas for the 1999–2000 period, the two loci were in linkage equilibrium. In 2003, after the translocation of the wild spawners, the tests with all the alleles revealed a significant departure from the HWE and a significant linkage disequilibrium ($P < 0.05$), whereas no significant differences were observed when the alleles were pooled into two categories according to their Atlantic and Mediterranean origins ($P > 0.05$). For the pooled allele data, subsequently lower values of D' were observed (Table 2).

The percentage of Atlantic alleles decreased significantly between the 1996–1997 period and the 1999–2000 period ($P < 0.01$) and remained similar in 2003. The distributions of the hybrid index score were significantly different between 1996–1997 period and both 1999–2000 and 2003 samples ($P < 0.01$) with a strong decrease from 40% to 0% of the potentially ‘pure’ Atlantic trout and an increase from 56% to 80% of the rate of genetically admixed trout. Between 1999 and 2000 period and 2003, the distributions of the hybrid index score remain similar (Fig. 2).

At sector B2, for both samples from 1996 to 1997 and in 2003 showed significant departures from HWE and significant linkage disequilibria ($P < 0.05$) (Table 2). The percentage of Atlantic alleles was significantly higher in the 1996–1997 sample than in the 2003 sample, however, standardised D' -values of 1.0 indicate maximum linkage between Atlantic and Mediterranean alleles in both samples. The distributions of the hybrid index score differ significantly ($P < 0.01$) between both 1996 and 1997 and 2003 samples with a strong decrease in the pure Atlantic individuals from 91% to 3%, whereas the percentage of pure Mediterranean individuals increased from 0% to 76% (Fig. 2).

Site C

The same temporal patterns of departures from HWE and linkage equilibrium were observed at both

Table 2. Standardised linkage disequilibrium coefficients (D') and results of tests for departures from Hardy–Weinberg (HWE) and genotypic linkage equilibrium (LD) considering all alleles and with hatchery and native specific alleles pooled.

	All alleles data		Pooled allele data		
	HWE	LD	HWE	LD	D'
SITE A					
1995	**	**	**	**	0.79
2000	NS	**	NS	**	0.76
2006	NS	NS	NS	NS	0.50
SITE B					
Sector B1					
1996–1997	NS	*	NS	*	0.51
1999–2000	NS	NS	NS	NS	0.37
2003	*	*	NS	NS	0.08
Sector B2					
1996–1997	*	*	*	*	1.00
1999–2000	No fish				
2003	*	*	*	*	1.00
SITE C					
Sector C1					
1999	NS	NS	NS	NS	0.03
2004	**	**	**	**	0.75
2006	NS	**	NS	**	0.49
Sector C2					
1999	NS	NS	NS	NS	0.56
2004	**	**	**	**	0.71
2006	NS	**	NS	**	0.72

(NS: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$).

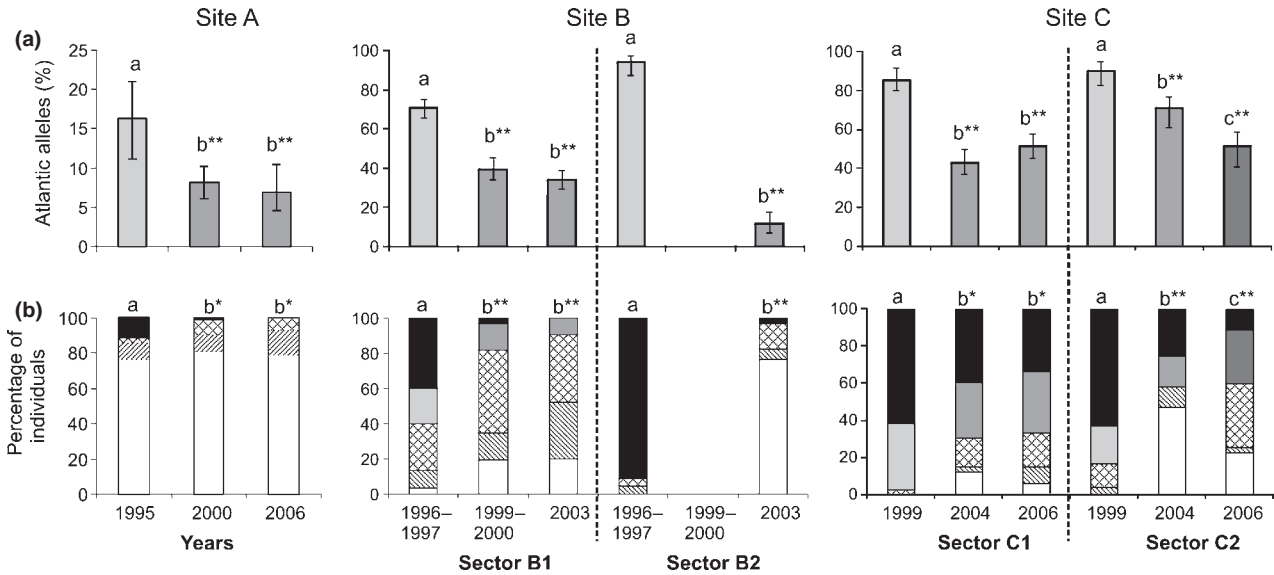


Fig. 2. Percentage of Atlantic alleles with 95%CL (a) and percentage of individuals assigned to the hybrid index scores (b) (□ 0; ▨ 1; ▩ 2; ▪ 3; ■ 4) calculated with the diagnostic loci in the three sites studied in the Dranse River System. Values having no common letter are significantly different with $P < 0.05$ (*) or $P < 0.01$ (**).

investigated sectors C1 and C2. For the 1999 samples, no significant departures from HWE ($P > 0.05$) and linkage equilibrium were detected. In 2004, the samples showed a significant deviation from the HWE ($P < 0.01$) and the two loci were not in linkage equilibrium. For the 2006 samples, the tests did not reveal any significant departures from HWE ($P > 0.05$) but the two loci were not in linkage equilibrium. However, the temporal pattern of the strength of linkage disequilibria (standardised D' -values) differed between the two sectors, i.e., at both sectors, the values increased from 1999 to 2004, but then decreased for 2006 sample of sector C1 and remained the same for 2006 sample of sector C2 (Table 2).

At sector C1, the percentage of Atlantic alleles decreased significantly between 1999 and 2004 ($P < 0.01$) and remained similar between 2004 and 2006 ($P > 0.05$). The distributions of the hybrid index score were significantly different between 1999 and both 2004 and 2006 samples ($P < 0.01$) with a decrease in the pure hatchery individuals (from 61 to 33%), a low increase in potentially pure Mediterranean trout (from 0 to 6%) and mostly an increase in admixed trout from 39% to 61%. Between 2004 and 2006, the distributions of the hybrid index score were similar ($P > 0.05$) (Fig. 2).

At sector C2, the frequency of Atlantic alleles decreased significantly between 1999 and 2004 ($P < 0.01$) and between 2004 and 2006 ($P < 0.01$). The distributions of the hybrid index score were significantly different between 1999 and 2004 and between 2004 and 2006 ($P < 0.01$). Between 1999

and 2004, the proportion of pure Atlantic trout decreased strongly from 62% to 25%, whereas that of the pure Mediterranean trout increased from 0% to 47%. But between 2004 and 2006, the percentage of Mediterranean trout decreases to 23%, whereas the frequency of admixed trout increased from 28% to 66% (Fig. 2).

Discussion

Because the three restoration strategies assessed here in the present study had been applied to three different situations, a direct comparison of their efficiencies is not meaningful. Nevertheless, the results obtained for each situation monitored can be used to improve the management aimed at conserving the native Mediterranean brown trout gene pool in the studied area. Furthermore, the results obtained here may lead to some general recommendations for the fisheries managers and conservationists for conservation and restoration programmes of native salmonid stream populations.

What did we learn about the three monitored situations?

The three strategies monitored in this study can be divided into two categories with different assumptions. The first one represents a passive approach, i.e., 'just let time do the job' by creating genetic refuges assuming that the standing population will purge 'itself' by selection against carriers of non-native alleles. The second one consists of an active intervention (i.e., translocation of wild spawners or stocking

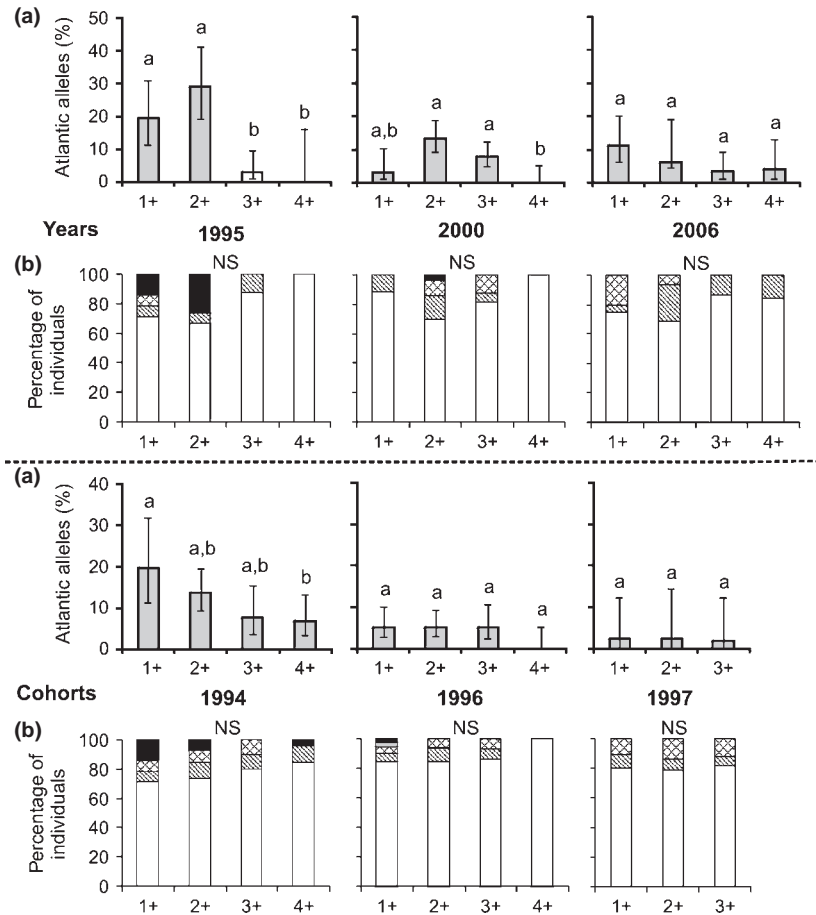


Fig. 3. Percentage of Atlantic alleles with 95%CL (a) and percentage of individuals assigned to the hybrid index scores (b) (□ 0; ▨ 1; ▩ 2; ◻ 3; ■ 4) calculated with the diagnostic loci in the site A for each age-classes for the years 1995, 2000 and 2006 and the cohorts 1994, 1996 and 1997. Values having no common letter are significantly different ($P < 0.05$). NS = no significant ($P > 0.05$).

with native trout) replacing the stocking with non-native fish to replace non-native populations by new native self-sustainable populations.

The results showed that the three strategies carried out had several detectable effects on the standing populations.

The establishment of the genetic refuges in both A and B1 sites caused, respectively, in 5 and 3 years, fast and significant decreases of the percentage of non-native Atlantic alleles. These decreases of Atlantic alleles can be mostly explained by the disappearing of the pure non-native Atlantic trout after stopping the stocking with Atlantic trout. Indeed, after this direct initial effect linked to the stopping of hatchery releases, the percentage of the Atlantic alleles in the samples influenced by the genetic refuge strategy remained stable over the monitored time. Yet, as is indicated by the decay of the departures of HWE and linkage equilibrium between diagnostic loci in over a short time span, it is indicated that the proportion of Atlantic alleles that has introgressed via recombination into the gene pool of the native trout may not be further reduced by this passive strategy.

These findings are in contrast to the results observed by Araguas et al. (2008) in the Mediterranean

populations in the eastern Pyrenees, which did not detect genetic differences (rate of hatchery introgression, deviation from HWE or linkage disequilibrium) in the samples before and after the establishment of genetic refuges.

Two nonexclusive main hypotheses can be proposed to explain the rapid disappearance of the pure non-native trout after the stocking was ceased. First, the Atlantic domestic trout could have a poor ability to self-sustain in a mountain stream inhabited by a native strain. Second, the fishery activities, maintained in both concerned sites, could allow the removing of the domestic trout by anglers. Indeed, several studies showed selective angling of domesticated introduced trout (García-Marín et al. 1998; Mezzera & Largiadèr 2001; Champigneulle & Cachera 2003; Heggenes et al. 2006). Moreover, according to the age-maturity relationship in these populations (Champigneulle et al. 2003) and the fast growth of the trout in the area studied (Champigneulle & Caudron unpublished data), the legal catch size of 25 cm was adequate to remove a majority of domestic fish at 2+ years during the angling season before the spawning period.

In the sector B1, inhabited by exogenous trout, the translocations of the Mediterranean spawners, in

addition of the genetic refuge strategy, showed a very low contribution to the wild population (apparition of only two new Mediterranean alleles) and they did not change significantly the introgression rate and the proportion of the potentially pure Mediterranean individuals in the population in comparison with the genetic refuge period. In this situation where the area was inhabited by a self-sustained population of Atlantic trout, the strategy of wild spawners translocation appears to have failed to improve the restoration of the Mediterranean native gene pool than the genetic refuge alone. This absence of contribution could have been *a priori* influenced by insufficient time period between the translocation carried out in 1999–2000 and the sampling in 2003. Indeed, bad hydrological conditions over the monitored time like flows during the spawning or hatching periods could have compromised strongly the survival of eggs and the natural recruitment (Jensen & Johnsen 1999; Spina 2001; Cattaneo et al. 2002) and in particular in this fast-flowing mountain stream. However, two observations are not in support of this explanation for the result obtained for sector B1. First, in agreement with the age–maturity relationship (Champigneulle et al. 2003) and proved by the scale analysis realised for this study, two different cohorts (2000 and 2001) of the F1 offspring of the translocated spawners had been sampled in 2003. Thus, it seems to be unlikely that two successive cohorts issued from wild spawners have been affected by a low survival rate, whereas at the same time, the electrofishing survey revealed a higher density of trout in 2003 than in the 1999–2000 period. Secondly, the results for sector B2 located immediately upstream of the sector B1 indicated that the same cohorts derived from the translocated spawners contributed strongly to the wild population sampled in 2003, and thus, indicated that the natural recruitment during the monitored time has been efficient.

At both sites, additional monitoring of the contributions of the translocated spawners to the next offspring generations of the wild population would have allowed to validate this explanation. Unfortunately, the scientific assessment of this strategy had to be stopped because fishery managers decided to change again their strategy and to stock this area with Mediterranean fry starting in 2004.

The stocking strategy with Mediterranean fry evaluated in the site C resulted in a marked and rapid decrease (in one generation) in the frequency of Atlantic alleles in the wild population. As for the two other strategies, this result could be also explained by the disappearance of the pure Atlantic trout released once the stocking with Atlantic domesticated fry have been stopped. The apparition of eight new Mediterranean alleles and the increase in the proportion of pure

Mediterranean trout in the wild population after only 4 years of stocking with Mediterranean fry suggest that the Mediterranean fry stocked indeed contributed to the increase in the Mediterranean alleles rate in the sector C2. This argument is supported by the results observed in the sector C1, which remained unstocked with Mediterranean fry over the study period, but which has been indirectly influenced by Mediterranean fry stocked upstream. In this sector, the reduction in the Atlantic allele fraction was slower, the pure Atlantic individuals persisted longer, the proportion of potentially pure Mediterranean trout was lower and less new Mediterranean alleles appeared over time than in the sector C2.

These results are in agreement with those obtained for the Ugine River, which is another stream of the Dranse River system, where the stocking with the same wild Mediterranean strain resulted in a significant reduction in the proportion of Atlantic alleles in the wild population and in the establishment of a self-sustaining population (Caudron et al. 2006). Other field studies (Hansen et al. 2006; Vincenzi et al. 2008) showed that introductions of native nonadmixed individuals can successfully establish new populations or restore an admixed brown trout population.

With the exception of site B2, where no fish were present 3 years after the stopping of the stocking with Atlantic trout, the results indicate that no strategy had been fully successful in restoring a nearly pure native population over the monitored time. Indeed, at sector B2, stopping the stocking with Atlantic trout caused the disappearance of the Atlantic population. That suggests that this non-native population had not been self-sustaining and had been completely maintained by stocked Atlantic individuals. In this particular situation, the wild spawners translocated were able to establish a new self-sustaining native population in a river section, where the natural recruitment of introduced Atlantic trout failed. A similar result has been obtained in upstream part of the Borne River, another mountain stream in the French Alps (Caudron, unpublished data). We speculate that the native trout, which colonised the rivers of this area about 10,000 years ago, is particularly adapted to the hydrologically unstable conditions of headwater mountain streams in this area or that the introduced Atlantic trout lost its potential to persist under these conditions because of 30 generations of domestication in a hatchery environment. Compatible with our explanation, Champigneulle et al. (2003) reported that native Mediterranean fish used several kinds of unusual microhabitat to spawn in a headwater mountain stream, whereas stocked Atlantic trout spawned in a single typical habitat. In any case, our results are compatible with a concept of local adaptation predicting that, in general, local populations are better

adapted to their particular environmental conditions than introduced individuals and that the artificial recolonisation is more successful when the donor zones are nearby and ecologically similar (Hindar et al. 1991; Young 1999; Reisenbichler et al. 2003; Utter 2004).

This also suggests that the restoration strategies should be more effective when there are no self-sustained non-native populations inhabiting the stretch of river concerned. This hypothesis is supported by the results of a recent experiment in another river consisting in the translocation of 1600 native Mediterranean trout of various year classes. The aim was to establish self-sustained population in an isolated large, 10 km long stretch of the river inhabited by a nonfunctional domesticated Atlantic population (Caudron et al. 2009). Indeed, in this case, the wild translocated trout created a functional and large population and the percentage of Atlantic alleles decreased drastically from 100% to <10% between 2004 and 2008, respectively, before and after the translocation of wild trout (Caudron & Guyomard unpublished data).

Genetic introgression between Atlantic and Mediterranean trout

An important finding of our study was that in all three situations monitored, the proportion of hybrid individuals between both non-native Atlantic and native Mediterranean trout increased showing that the introgression of the native gene pool by non-native gene continued. The active strategies carried out by managers led to intraspecific introgression between both non-native Atlantic and native Mediterranean strains in the upper isolated parts of river. The temporal evolution of the standardised linkage coefficients (D' -values) in the different monitored sectors is also in accordance with this interpretation. Indeed, the strong reduction in the D' -values observed for the 2006 samples of the site A and C1, respectively, after 11 years of genetic refuge and 6 years of stocking with Mediterranean fry, indicates successful reproduction of later generation hybrids. For sector B1, the nonsignificant departures from linkage equilibrium together with the low samples of the B1 D' -values observed for the period influenced by the genetic refuge and the translocation strategy (Table 2), respectively, suggest a nearly completely mixed and randomly mating population. Finally, only one sector, B2, showed a maximum value of D' (= 1.0), which suggests for this sector that the interbreeding has not yet substantially gone past the F1 generation.

Low values of D' generally suggest that the observed proportion of non-native Atlantic alleles may not be further reduced by natural selection. And

in such situations, managers will be confronted with the question, what is an acceptable level of introgression. The hybrid status of population is a common issue for the conservation programmes of native salmonids (Allendorf et al. 2001, 2004; Largiadèr 2007). And, managers are often confronted with a big dilemma between letting non-native populations in the upper parts of river threatening by gene flow the downstream native populations or trying to carry out active restoration strategy with the risk of creating interbred buffer populations and/or the lose part of the local genetic variation by replacing introgressed populations. Clearly, the knowledge is insufficient to assist managers in their decisions, and many additional studies are needed to monitor at long term about the efficiency of the programmes for the genetic conservation and restoration of freshwater autochthonous trout populations.

Conservation implications

Taking into account these present findings, some recommendations can be formulated to improve the future conservation programmes of native brown trout populations threatened by non-native populations.

The first conservation measure seems obvious: stop all introductions of non-native fish in a hydrographic network inhabited by native populations to reduce the potential source of further introgression. This strategy allowed to conserve as much as possible the local native genetic diversity but nevertheless, according to previous studies (Poteaux et al. 1998; Araguas et al. 2008) and the present results, it was not efficient to collapse the introgression of the Mediterranean native gene pool by domesticated Atlantic strains. Moreover, according to Araguas et al. (2009), the effectiveness of the genetic refuge strategy for protecting native gene pool depends also on its acceptance by anglers and fisheries managers to avoid illegal stocking in protected area.

When demography of the native population is able to support the fishing pressure, it can be proposed to maintain the recreational fisheries. Indeed, the apparently selective angling of domesticated trout (García-Marín et al. 1998; Mezzera & Largiadèr 2001; Champigneulle & Cachera 2003; Heggenes et al. 2006) could limit the genetic impact of the stocking in native populations.

The present results suggest to wait at least 3–5 years before to carry out an active restoration strategy like translocation of wild individuals or stocking with native fry in addition of the genetic refuge. This time can lead, in some part of river, to the disappearance of non-native populations artificially sustained by stocking. These locations can then be restored in priority by managers.

In all the Northern French Alps territory as in the present study area, habitat fragmentation by both artificial and natural obstacles has strong impact on the genetic population structure of the population and especially also on the spatial distribution of native and the introduced trout (Caudron 2008; Caudron et al. 2009). Because habitat fragmentation seems to be an important factor influencing the success of the restoration programmes, it is very important, before deciding to carry out a management intervention, to know precisely the spatial distribution throughout the entire managed territory of the non-native and native populations and the degree of isolation between each population.

In the case of persistence of self-sustained non-native populations despite the stopping the stocking over several years, in agreement with the present findings, we do not recommend to carry out additional restoration strategies, which will introduce native trout in stretches of river inhabited by functional non-native populations. We suggest to monitor over time the genetic effects of the isolated non-native populations on the neighbouring native populations to better appreciate long term impacts.

In some case, when the parts of river inhabited by non-native Atlantic populations is not too long (<3 km for instance), the removal of the non-native trout by successive electrofishing can be proposed. Nevertheless, several field experiments conducted in Rocky Mountain streams (Larson et al. 1986; Moore et al. 1986; Thompson & Rahel 1996; Kulp & Moore 2000; Shepard et al. 2002; Peterson et al. 2004; Meyer et al. 2006) showed that this strategy was laboriously and expensive because to be effective, multiple consecutive years of suppression are needed. Moreover, the removal electrofishing is inefficient in a river with complex habitats such as deep pool, unclogged substrates, stream cover and woody debris (Riley & Fausch 1992; Thompson & Rahel 1996; Kulp & Moore 2000).

Finally, collaborative and multidisciplinary approach involving scientists and managers need to be developed for the brown trout in Europe such as those developed for native salmonid populations in North America. For example, the monitoring of a restoration programme of Steelhead showed that the use of local origin could increase the short-term abundance of depleted population and that could also cause long-term genetic change in the population (Carofino et al. 2008). Hilderbrand (2002) indicated by simulating that stocking 10% of the carrying capacity with adults in a one time or with age-0 fish or subadults over multiple years may be an effective conservation strategies to achieve long-term population persistence of resident Cutthroat trout (*Oncorhynchus clarki*). The simulation of supplementation strategies to conserve the coast

Coho salmon (*Oncorhynchus kisutch*) populations revealed that the conservation hatchery programmes provided no significant increase in the abundance and caused significant ecological and genetic risks (Oosterhout et al. 2005). Several studies developed models to predict translocation success of Cutthroat trout based on the stream habitat to reduce uncertainty in the management decision (Harig & Fausch 2002; Young et al. 2005). Fausch et al. (2008) developed a conceptual framework of the joint trade-off of invasion and isolation threats to help managers make decisions to install or remove barriers to conserve native Salmonids.

We are convinced that this combination of field monitoring programmes and modelling approaches need to be applied for the conservation of native brown trout populations to control the effects of different management scenario on the wild population, to provide managers predictive tools for the successful evaluation of different restoration strategies and finally to refine restoration programmes.

Acknowledgements

This work was supported by funds from the Haute-Savoie Departmental Federation of Fisheries and the National Federation of French Fisheries. We thank B. Riva (fishing guard of the local fisheries association) for his contribution to the field work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Allele frequencies observed at the Str591 and Str541 loci in the studied samples. In bold, alleles of Mediterranean origin.

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