Journal of Fish Biology (2006) **69** (Supplement A), 127–139
doi:10.1111/j.1095-8649.2006.01156.x, available online at http://www.blackwell-synergy.com

Assessment of restocking as a strategy for rehabilitating a native population of brown trout *Salmo trutta* L. in a fast-flowing mountain stream in the northern French Alps

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(Received 6 July 2005, Accepted 7 April 2006)

An attempt was made to extend the area of distribution of a native population of brown trout *Salmo trutta* belonging to a Mediterranean lineage (ML), which has maintained itself in the Dranse d'Abondance, a fast-flowing alpine stream (Haute-Savoie, France), despite several decades of intensive restocking with brown trout derived from the Atlantic lineage (AL). This was done by releasing an ML component into the predominantly AL population still present on the Ugine, the main tributary of the Dranse d'Abondance. This strategy of rehabilitation restocking was tested using fluoro-marked juveniles produced from a captive breeding stock derived from the wild Dranse d'Abondance ML stock. Samples of 0+ year fish were collected over the period 1995–2003 in order to assess the impact of the restocking. Percentages of fluoro-marked otoliths revealed significant contributions of ML restocking in the 0+ year autumnal standing population, with levels ranging from 34·3 to 61·4%. The change in the genetic characteristics of the 0+ year population produced by natural recruitment was monitored by analysing the unmarked subjects. Frequencies observed at two microsatellite loci revealed a considerable rise (from 0 to 60%) in the level of Mediterranean alleles in the natural 0+ year population since the introduction of restocking using ML individuals.

Key words: conservation; genetics; native strain; otolith marking; restocking; *Salmo trutta*.

INTRODUCTION

Population genetics studies of natural populations of brown trout *Salmo trutta* L. in France reveal that there are two main different lineages, the Atlantic lineage (AL) in the Atlantic catchment area and the Mediterranean lineage (ML) in Mediterranean rivers. These two lineages substantially differ in their mtDNA haplotypes (Bernatchez *et al.*, 1992) and their allozyme and microsatellite allele frequencies (Guyomard, 1989*a;* Estoup *et al.*, 2000; Launey *et al.*, 2003*a*). The French north alpine hydrographic catchment area belongs

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to the Mediterranean basin and harbours the ML lineage (Guyomard, 1989*a*; Launey *et al.*, 2003*a*). These ML populations must be considered as native populations since almost all of the stocking operations have been done with hatchery stocks of Atlantic origin until now (Krieg & Guyomard, 1985; Launey *et al.*, 2003*a*).

Several studies (Barbat-Leterrier *et al.*, 1989; Beaudou *et al.*, 1994; Poteaux *et al.*, 1998) have shown that the AL has replaced the ML originally present in many streams of the Mediterranean basin. In contrast, in some Mediterranean streams, despite similar stocking practices, native populations showing little introgression by the AL (Barbat-Leterrier *et al.*, 1989; Largiader *et al.*, 1996; Launey *et al.*, 2003*a*) are still present. In order to ensure the long-term preservation of populations that display low introgression rates, conservation measures, such as the stopping of massive stocking with AL brown trout, have been gradually introduced. In addition to this strategy of conserving the autochtonous populations, ML population restoration strategies can be undertaken in nearby areas that have a similar environment but which are no longer colonized by ML. The ultimate goal is to expand the spatial distribution of some of the native ML populations that are still present and to strengthen them.

This latter approach has been tried out on the Ugine, a tributary of the Dranse d'Abondance, a typical mountain stream system located in the northern French Alps. The present study reports on a first assessment of this population restoration strategy based on stocking with fish from a nearby native population.

MATERIALS AND METHODS

LOCATION AND CHARACTERISTICS OF THE SITE INVESTIGATED

The study was carried out on the fast-flowing Ugine River, the main tributary of the Dranse d'Abondance located in the northern French Alps, in Haute-Savoie (Fig. 1). This zone belongs to the Mediterranean catchment area. The Dranse d'Abondance is one of the three upstream branches of the Dranse system, which is the second largest affluent of Lake Geneva and belongs to the Mediterranean catchment. The Ugine, which is 11 km in length, has its source at an altitude of 1600 m. It has a mean slope of 10%. Its catchment area covers 31 km^2 and ranges in altitude from 630 to 2220 m. It has the typical geomorphologic characteristics of a middle-altitude mountain stream in the Alpine zone. The zone studied covers the median section of the Ugine, which includes most of natural production zones of this stream. The zone studied is separated from the main stream of the Dranse d'Abondance by the insurmountable barrier of a hydroelectric dam, located in the lower part of the Ugine.

RESTOCKING PRACTICES

Recent genetic studies (Largiader *et al.*, 1996; Launey *et al.*, 2003*a*) have demonstrated that most of the main stream of the Dranse d'Abondance and several of its tributaries harboured a large, nearly pure Mediterranean population, despite intensive stocking over several decades with fish originating from the AL. The zone of low introgression has been classified as a sanctuary and has not been restocked since 1996.

Wild spawners (150 males and 150 females) were caught at three places (Fig. 1) in this area and used to produce separate full-sib families. Only the 98 families obtained

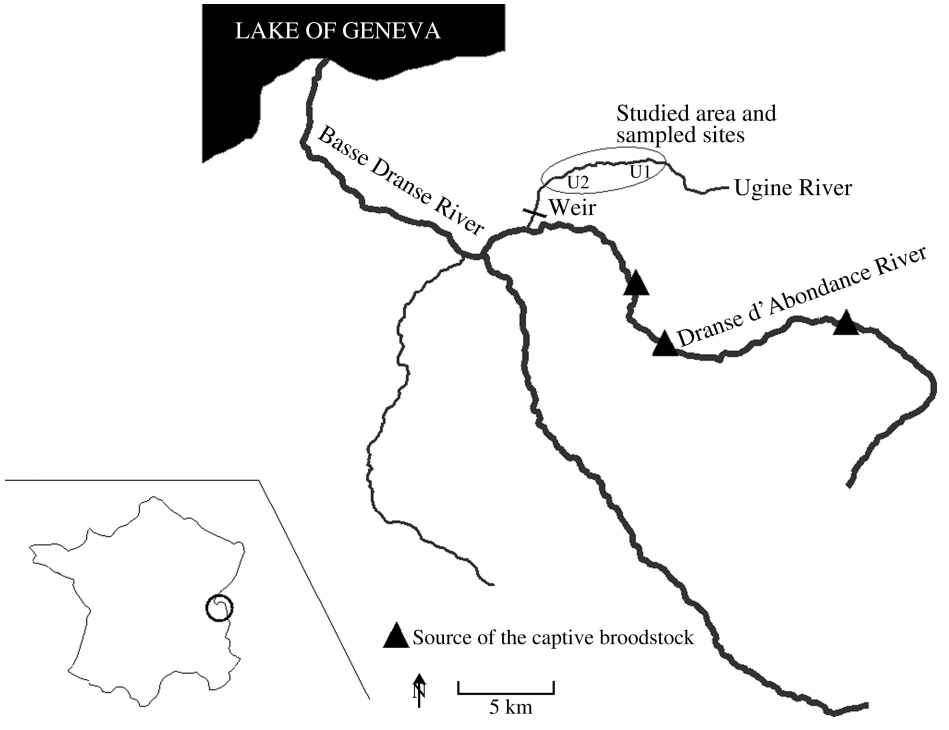

FIG. 1. Location of the catchment area of the Ugine and the U1 (upstream) and U2 (downstream) sections studied.

from parents (*i.e.* 98 females and 98 males) showing Mediterranean genotypes at three diagnostic markers, *Str54-1*, *Str59-1* and *Str79-1* (Estoup *et al.*, 2000; C. R. Largiader, unpubl. data) were kept to found the ML captive breeding stock.

Until 1998, the Ugine River was massively and exclusively restocked during several decades with AL fish. In 1995 and 1998, small numbers of ML brown trout fingerlings were also released. Since 1999, brown trout restocking methods in the Ugine have been changed (Table I), and all the fry released have been derived from the captive ML breeding stock. The numbers of ML fish introduced each year was *c.* a quarter of the number of annually released AL fish (Table I).

MARKING THE INDIVIDUALS RELEASED AND SAMPLING

In 1995, all the AL fry (44 000) released into the Ugine at early stage (Table I) were otolith 'fluoro-marked' by immersion in a saline solution (50 g l^{-1} NaCl) of 10 g l^{-1} tetracycline hydrochloride (TCHC) for 3·5 min (Champigneulle & Rojas Beltran, 2001). In the same year, the 1 100 ML fingerlings released were marked by removing the adipose fin.

Since 1999, all the ML fry released have been fluoro-marked by immersion in a 100 mg l^{-1} solution of alizarin red S (ARS) during 3 h (Caudron & Champigneulle, 2006).

Samples of 0+ year fish were taken in autumns of 1995, 2000 and 2003 by electrofishing in the zone being studied. In 1995, a section (U1) of 500 m was sampled. In 2000 and 2003, in addition to U1, a second section (U2) of 500 m located downstream from U1 (Fig. 1) was sampled. U1 and U2 together correspond to a 1 km stretch of river that can be considered to be representative of the population present in the Ugine River. Indeed, the sampling was carried out continuously over the electrofishing area in order to obtain the better representativity of the river stretch.

TABLE I. Quantity and source of the restocking brown trout released into the Ugine River between 1988 and 2003

	Atlantic lineage (AL) fish			Mediterranean lineage (ML) fish			Total	
Years	Fry 2–3 cm L_T	Fry 4–5 cm L_T	Fingerling	Fry 2–3 cm L_T	Fry 4–5 cm L_T	Fingerling	AL	ML
1988	55 000	20 000					75 000	0
1989	25 000	15 000					40 000	0
1990	27 000	19 500					46 500	0
1991	25 000	19 500					44 500	0
1992		50 000					50 000	0
1993	30 000	25 000					55 000	0
1994	25 000	23 000					48 000	0
1995	**30 000**	**8 000**	**6 000**			*1 100*	**44 000**	*1 100*
1996	30 000						30 000	0
1997	40 000	4 000	200				44 200	0
1998	17 800					300	17 800	300
1999				**12 000**			0	**12 000**
2000					**11 000**		0	**11 000**
2001				**9 000**	**1 900**		0	**10 900**
2002				**9 000**	**2 000**		0	**11 000**
2003				**9 000**	**2 200**		0	**11 200**

In bold, fish marked by otolith fluoromarking; in italic, fish marked by removing the adipose fin. L_T, total length.

The age of each fish was determined by scalimetry in order to check that they belonged to the 0+ year cohort.

ANALYSIS OF OTOLITHS

For each of the 0+ year fish sacrificed, the head was dissected in order to access the sacculi containing the otoliths (sagittae). Otoliths were removed, mounted on glass slide with a thermoglue (Crystalbond Aremco adhesive number 509) and polished to expose the nucleus (Caudron & Champigneulle, 2006).

In order to detect the fluorescent mark and estimate the proportions of natural (hereafter named unmarked) and introduced (hereafter named marked) 0+ year fish, each slide was observed under a Zeiss Axioskop 40 epifluorescence microscope (mercury vapour lamp HBO50) using a Zeiss number 15 filter and Zeiss number 09 filter for ARS and TCHC, respectively.

GENOTYPING

Fin clips were taken from each 0+ year fish sampled and kept in 95% ethanol. Genotyping was carried out, on one hand, to determine the change in the genetic characteristics (level of introgression and genotype composition) of the natural fraction of juveniles *in situ* and, on the other hand, to measure the degree of genetic similarity between the natural 0+ year fish and the individuals introduced in the previous years.

Samples were genotyped with two microsatellite markers, *Str54-1INRA* and *Str59-1INRA*, which are located on two distinct linkage groups (BT2 and BT7, K. Gharbi, unpubl. data). The AL or ML origin of the different alleles found at two loci was unambiguously

determined by the combination of the information provided at these two loci and at two adjacent markers, *Str54-2INRA* and *Str59-2INRA*. The rationale of this method is explained in Estoup *et al.* (1999) and the diagnostic nature of these two sets of markers has been validated in numerous Mediterranean populations from France, Italy, Greece and Spain and from farmed strains used locally (Estoup *et al.*, 2000; Launey *et al.*, 2003*b*; R. Guyomard, unpubl. data;). In practice, the genotyping of the two additional loci, *Str54-2INRA* and *Str59-2INRA*, is restricted to a sub-sample of natural and fish-farmed individuals in order to determine the genetic origin (Mediterranean *v.* Atlantic) of the alleles observed at the *Str54-1INRA* and *Str59-1INRA* loci. Genotyping at *Str54-2INRA* and *Str59-2INRA* and allele validation in sub-samples from the Dranse d'Abondance basin were performed in previous studies (Estoup *et al.*, 2000; Launey *et al.*, 2003*b*; R. Guyomard, unpubl. data). DNA from each individual was purified in an extraction buffer containing Chelex® (Estoup *et al.*, 1996).

The *Str54-1INRA* and *Str59-1INRA* markers were co-amplified by polymerase chain reaction (PCR) where 3' primers were marked with the Fam and Tamra fluorochromes, respectively. The PCR amplifications, separation and visualization of the PCR products were carried out according to Launey *et al.* (2003*b*), with a denaturing phase of 5 min at 96° C, five cycles consisting of 30 s at 96° C, 30 s at 54° C, 1 min at 72° C, 20 cycles of 30 s at 95° C, 30 s at 54° C, 30 s at 72° C and a final extension phase of 5 min at 72° C.

STATISTICAL ANALYSES

The 95% CL of the contributions of the marked and unmarked fry were calculated from Beyer's (1986) tables. Statistical differences in proportions of marked and unmarked fish among sections and years were tested with a χ^2 test. Statistical significance of deviations from expected Hardy–Weinberg proportions, linkage disequilibria and genetic differentiations were tested using the 'Hardy–Weinberg', 'Genotypic disequilibrium' and 'Population differentiation' options of GENEPOP (Raymond & Rousset, 1995). The introgression rate was estimated by the average frequencies of Atlantic alleles at the *Str54-1INRA* and *Str59-1INRA* loci (*i.e.* number of Atlantic alleles at *Str54-1INRA* and *Str59-1INRA* found in a sample divided by four times the number of individuals analysed in this sample). Thereafter, the term introgression refers to the introduction of alien genes and their incorporation into the native gene pool.

RESULTS

CONTRIBUTION OF RESTOCKING AT STAGE 0+ YEAR IN 1995, 2000 AND 2003

In 1995, despite the massive restocking, mainly consisting of AL (introduction of 44 000 AL fry and of 1 100 ML fingerlings), the population of juveniles sampled during the autumn in the U1 section was mainly (85·7%) derived from natural recruitment (Fig. 2). In this cohort, the introduced AL (marked with TCHC) and ML individuals (marked by removing the adipose fin) represented only 2·9 and 11·4% of the population of juveniles present in the autumn, respectively.

In the U1 section, the proportion of marked subjects did not differ significantly ($P > 0{\cdot}05$) between 2000 (48·1%) and 2003 (61·4%) and both of them were significantly ($P < 0{\cdot}01$) higher than in 1995 (14·3%).

In the U2 section, the percentages of marked 0+ year fish were similar ($P > 0{\cdot}05$), reaching *c.* 35% in each of the cohorts studied.

Globally, the contributions of the ML (marked) fry to the 2000 and 2003 cohorts (respectively, 40·3 and 46·3%) were significantly higher than those of

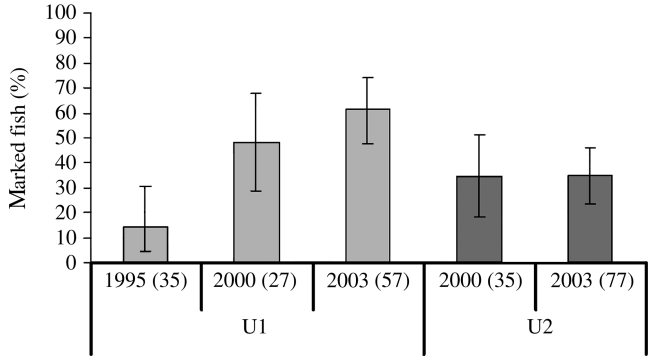

FIG. 2. Contribution of restocked (percentage ± 95% CL marked) brown trout to the 0+ year autumnal population in the U1 and U2 sections located in 1995, 2000 and 2003 (n = sample size).

marked fry in the 1995 cohort (14·3%) ($P < 0{\cdot}05$) and did not change significantly between 2000 and 2003.

CHANGE IN THE GENOTYPE COMPOSITION AND INTROGRESSION RATE IN THE NATURAL POPULATION

Genetic analysis identified three Atlantic alleles (146, 150 and 152) and nine Mediterranean alleles (166, 170, 176, 190, 192, 194, 196, 198 and 200) at *Str59-1INRA*, two Atlantic alleles (130 and 132) and one Mediterranean allele (136) at *Str54-1*. The 0+ year sample derived from natural reproduction in 1995 was fixed for the Atlantic alleles 150 and 132 at *Str59-1* and *Str54-1*, respectively (see Appendix for the allele frequencies).

Between 1995 and 2003, the genetic composition of the 0+ year individuals derived from natural recruitment changed considerably with a very marked increase in the proportion of Mediterranean alleles (Fig. 3).

A multi-locus test for Hardy–Weinberg expectations was significant in the U2 sample for the year 2000 ($P < 0{\cdot}05$) only. This apparently resulted from the

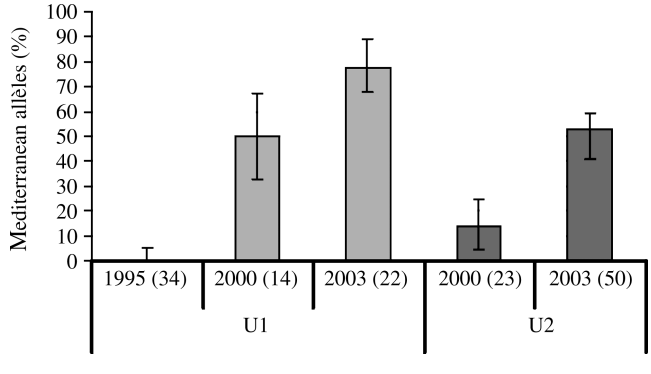

FIG. 3. Percentage ± 95% CL percentage of the Mediterranean allele calculated at the *Str54-1INRA* and *Str59-1INRA* loci in the samples collected in 1995, 2000 and 2003 at stage 0+ year in the U1 and U2 sections (n = sample size).

occurrence of two pure ML individuals, which exhibited ML alleles only at both loci since the test was no longer significant when these two individuals were discarded. The multi-population test for Hardy–Weinberg proportions, however, was not significant ($P > 0{\cdot}05$) and, thereafter, it was assumed that conformity to Hardy–Weinberg proportions was fulfilled in all samples. The two loci were in linkage equilibrium only in the sample from the U1 section in 2000, and the global test, carried out on all the samples, was very highly significant ($P < 0{\cdot}001$).

The samples of marked individuals released in 2000 and 2003 did not deviate significantly from the Hardy–Weinberg expectations ($P > 0{\cdot}05$) and were at linkage equilibrium ($P > 0{\cdot}05$). The frequencies of these samples did not significantly differ from each other ($P > 0{\cdot}05$) and were very similar to those observed in the source population (C. R. Largiader, unpubl. data).

The introgression rates showed similar pattern of change in both sections and decreased considerably over time. In the U1 section, the level of introgression within the natural population of juveniles (unmarked) decreased significantly between 1995 and 2000 ($P < 0{\cdot}001$), and between 2000 and 2003 ($P < 0{\cdot}001$), with values of 100, 50 and 23% for the years 1995, 2000 and 2003, respectively (Fig. 3). For the U2 section, the level of introgression was 83% in 2000 and decreased significantly ($P < 0{\cdot}001$) to 47% in 2003 (Fig. 3). Introgression rates were significantly lower ($P < 0{\cdot}001$) in the U1 than in U2 section in both 2000 and 2003.

DISCUSSION

BACKGROUND, ORIGINALITY AND OBJECTIVES OF THE STUDY

Several recent studies have reported or predicted a high risk of extinction of original native populations in many species of salmonids [Dowling & Childs, 1992, for *Oncorhynchus apache* (Miller); Young, 1999, for *Oncorhynchus* spp.; Giuffra *et al.*, 1996 and Crivelli *et al.*, 2000, for *Salmo marmoratus* Cuvier; Laikre *et al.*, 1999, for *S. trutta*; Hilderbrand, 2002, for *Oncorhynchus clarki* (Richardson)].

A recent Europe-wide overview (Laikre *et al.*, 1999) stressed the need to set up long-term management at a populational level rather than at species level to ensure the long-term survival of *S. trutta*. These authors also pointed out that little work has yet been carried out to test the effectiveness of various types of conservation strategies and of a management of intraspecies biodiversity in the brown trout.

The current project results from the discovery of a native ML brown trout population in the main part of the Dranse d'Abondance River (Largiader *et al.*, 1996; Launey *et al.*, 2003*a*) and, in contrast, the presence of a population highly introgressed by Atlantic stocks in the Ugine River. The initial situation in the Ugine was presumably the consequence of the repetitive and intensive stocking during nearly 100 years in a progressively damaged habitat. Indeed, the studies of Largiader *et al.* (1996) and Launey *et al.* (2003*a*) support the hypothesis of a strong impact of hatchery brown trout on the populations of several tributaries from Lake Geneva.

The first conservation measure introduced in this zone was to stop all restocking operations with non-native strains, which are thought to be one of

the main reasons underlying the disappearance of native populations of salmonids (Allendorf & Leary, 1988; Ferguson, 1989; Hindar *et al.*, 1991; Waples, 1991; Leary *et al.*, 1993; Hansen & Loeschcke, 1994; Largiader & Scholl, 1995; Allendorf & Waples, 1996; Crivelli *et al.*, 2000).

In order to restore the ML in the Ugine River, the following strategies were envisaged (used either separately or in combination): 1) the construction of fish ladders between the Dranse d'Abondance and the downstream Ugine, 2) the preliminary eradication of the non-native fraction (the AL fish in this case), a method that has sometimes been used in the U.S.A. (Harig *et al.*, 2000), 3) the transplantation of ML adults caught in the Dranse d'Abondance into the Ugine, 4) a temporary restocking with ML juveniles produced from a breeding stock derived from the ML population present in the adjacent Dranse d'Abondance River. It was decided to implement only this last strategy for the following reasons. A ML broodstock from the adjacent Dranse d'Abondance River was available. There was no evidence of the presence of a native ML brown trout population in the Ugine River. So there was no risk of lowering fitness by the introduction of an adjacent native ML population.

Many studies of the impact of AL restocking on the genetic characteristics of natural ML populations have been published (Guyomard, 1989*a*, *b;* Barbat-Leterrier *et al.*, 1989; Beaudou *et al.*, 1994; Largiader *et al.*, 1996; Poteaux *et al.*, 1998; Berrebi *et al.*, 2000*a*). As far as is known, the case of the Ugine River is one of the first in which the consequences of switching from AL-type to ML-type restocking, and the feasibility of restoring a functional native population to replace an AL population, have been investigated. Marking all the fry released made it possible to distinguish unambigously between introduced and natural juveniles in the local population, and to monitor the temporal changes of the genetic characteristics of the fraction derived from natural recruitment.

RESTOCKING WITH A WILD STRAIN: EFFECTIVENESS AND ABILITY TO INTRODUCE A FUNCTIONAL POPULATION

The main result observed was an increase of Mediterranean allele frequencies in the natural 0+ year population following restocking with ML individuals. This increase was already obvious in the 2000 cohort, even if only a small number of ML stocked juveniles had only been introduced on two occasions, in 1995 and 1998.

Two non-exclusive situations could account *a priori* for the observed change: 1) a natural migration from downstream or upstream and 2) the modification of stocking practices.

Situation 1. The possibility of a natural recolonization of the studied zone can be discarded for two reasons: 1) the presence of an impassable obstacle in the lower part of the Ugine River preventing brown trout migration from the Dranse d'Abondance River (Fig. 1); 2) the zone upstream from U1 harbours a very small brown trout population as revealed by recent surveys (November 2004). Moreover if such a ML spawner population would have existed upstream in the mid-1990s, it would be difficult to explain why it did not contribute to the 0+ year cohort of U1 section (located just downstream) in 1995 and strongly contributed to it in the subsequent years.

Situation 2. The most likely explanation is that some of the ML individuals introduced have established themselves and reproduced successfully, their contribution probably overweighting the natural recruitment. The marked reduction in Atlantic allele frequencies in the U1 section from 1995 to 2000 indicates that the 0+ year fish restocked in 1995 and in 1998 have become well established and contributed to the natural breeding. According to the age-maturity relationships in ML brown trout (Champigneulle *et al.*, 2003*a*), 0+ year males and females introduced in 1995 would have contributed to natural recruitment as soon as 1996 (stage 1+ year) and 1997 (stage 2+ year), respectively and until 1999. The male and female offspring of ML males introduced in 1995 could have also contributed to the natural reproduction and the increase of Mediterranean allele frequencies in 1998 and 1999, respectively, while the male offspring of ML females could have reproduced in 1999.

The reasons of the pronounced shift towards Mediterranean alleles between 1995 and 2000, despite the fact that stocked AL fish largely outnumbered ML ones over this period, cannot be identified here. The two stocks differ genetically with regard to two aspects, which could explain the apparent higher survival rate and reproductive success of ML fish: their geographic origin and their history of domestication. First, the ML strain used here has been founded with local native breeders. Local populations are assumed to be adapted to their particular environmental conditions (Hindar *et al.*, 1991, Reisenbichler *et al.*, 2003) and recolonization by artificial propagation is more often successful when the donor zones are nearby and ecologically similar (Reisenbichler, 1988; Rhodes & Quinn, 1999; Young, 1999; Utter, 2004). Secondly, the ML stock has experienced only one generation of domestication instead of 20–30 for the AL stock. There are accumulative evidences that domestication can affect gene frequencies (Ryman & Ståhl, 1980) and important traits such as survival, feeding and reproductive behaviour (Reisenbichler & McIntyre, 1977; Berg & Jorgensen, 1991; Ruzzante, 1994; Johnsson *et al.*, 1996; Champigneulle *et al.*, 2003*b*; Huntingford, 2004; Kostow, 2004) and that farm escapes can have adverse effects on the fitness of wild stocks (Fleming & Petersson, 2001; McGinnity *et al.*, 2003). These differences are assumed to increase with the number of generations of fish culture (Waples, 1999).

The effects of non-genetic factors such as the fish size at stocking (Hume & Parkinson, 1988) or interannual variation in stocking efficiency, however, cannot be excluded and common garden field experiments are required to evaluate the respective role of genetic background and environmental variations and to make conclusions on the superiority of ML genotypes.

MANAGEMENT IMPLICATIONS

This study, combining genetic analyses and demographic data, has demonstrated that the restoration of a functional ML population in the Ugine River was successfully achieved through restocking with fingerlings and fry from a recent captive ML stock derived from a nearby native population living in a comparable habitat. A similar rehabilitation programme was carried out on the threatened *S. marmoratus* in Slovenia. In this country, pure populations of *S. marmoratus* have been identified (Berrebi *et al.*, 2000*b*; Fumagalli *et al.*,

2002) and used to propagate *S. marmoratus* in fish-free isolated sections (Crivelli *et al.*, 2000). Preliminary results are encouraging since new pure populations of *S. marmoratus* have been established and their allele frequencies have increased in rivers where exotic fish are no longer stocked (A. Crivelli, pers. comm.).

In conclusion, rehabilitation stocking can be one of the strategies to reintroduce functional brown trout population from a neighbouring non-introgressed native population, to restore the area of distribution of original taxons of native brown trout at local scale, and thus to strengthen their conservation in the long-term.

The relatively high percentage of marked 0+ year fish observed in 2003, indicates that it is still too early to recommend the complete cessation of rehabilitation restocking of the Ugine River. Monitoring the percentage of marked juveniles and the allele frequencies in the natural recruitment component of the 0+ year population should be continued. This information is required to check that the new ML population is self-sustaining and to decide when rehabilitation restocking can be stopped.

This study is part of an INTERREG IIIA ALCOTRA programme: 'Identification, preservation and rehabilitation of populations of native trout in the Val d'Aoste and in Haute-Savoie.' We would like to thank M. Andriamanga (INRA Jouy en Josas) who carried out the genetic analyses, C. Largiader (Berne University), S. Launey (INRA Jouy en Josas) and B. Riva for their contribution to the initial phase of foundation of the ML breeding stocks.

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APPENDIX. Allele frequencies observed at the *Str59-1* and *Str54-1* loci in the six samples. In Italic, total of the 2000 and 2003 samples of otolith marked individuals; in bold, alleles of Atlantic origin

		Population				
Locus	Marked	U1-1995	U1-2000	U2-2000	U1-2003	U2-2003
Str59-1						
(*n*)	*79*	31	14	23	22	50
146	***0·0063***	**0·0000**	**0·0000**	**0·0217**	**0·0000**	**0·0400**
150	***0·0886***	**1·0000**	**0·4286**	**0·8043**	**0·2273**	**0·4100**
152	***0·0000***	**0·0000**	**0·0714**	**0·0000**	**0·0227**	**0·0100**
166	*0·0759*	0·0000	0·0357	0·0435	0·0000	0·0300
170	*0·1582*	0·0000	0·1786	0·0217	0·0909	0·1500
176	*0·0127*	0·0000	0·0000	0·0000	0·0455	0·0000
186	*0·0253*	0·0000	0·0000	0·0000	0·0000	0·0100
190	*0·0253*	0·0000	0·0000	0·0000	0·0000	0·0100
192	*0·0949*	0·0000	0·0714	0·0435	0·2045	0·0700
194	*0·3481*	0·0000	0·0357	0·0000	0·1364	0·1600
198	*0·0823*	0·0000	0·1786	0·0435	0·2727	0·0900
200	*0·0823*	0·0000	0·0000	0·0217	0·0000	0·0200
H_{nb}	*0·8213*	0·0000	0·7672	0·3536	0·8214	0·7754
H_{obs}	*0·7975*	0·0000	0·8571	0·2609	0·9091	0·7400
Str54-1						
(*n*)	*79*	31	14	23	22	50
130	***0·0000***	**0·0323**	**0·0000**	**0·0435**	**0·0000**	**0·0000**
132	***0·0380***	**0·9677**	**0·5000**	**0·7826**	**0·2045**	**0·4900**
136	*0·9620*	0·0000	0·5000	0·1739	0·7955	0·5100
H_{nb}	*0·0735*	0·0635	0·5185	0·3633	0·3330	0·5048
H_{obs}	*0·0506*	0·0645	0·5714	0·2174	0·2273	0·3800
H_{mean}	*0·4474*	0·0317	0·6429	0·3585	0·5772	0·6401
N_{allele}	*6·5000*	1·5000	4·5000	5·0000	4·5000	6·5000

n, sample size; H_{nb}, expected, unbiased heterozygosity; H_{mean}, mean, expected, unbiased heterozygosity; N_{allele}, mean number of alleles per population.