

APPLIED ISSUES

Early effects of the strategies of creating a genetic refuge and direct translocation for conserving and restoring populations of native brown trout

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SUMMARY

1. The conservation of salmonid inter- and intra-specific diversity is a well-known challenge, and general management guidelines and conservation processes are available. However, research demonstrating the outcomes of practical conservation actions is largely lacking.

2. We monitored the spatiotemporal genetic and demographic evolution of a native Mediterranean brown trout population in a river in the French Alps to assess the efficacy and early effects of genetic refuge (i.e. cessation of stocking) and wild trout translocation strategies. We also studied the use of angling as a tool to limit the introgression of the wild standing population.

3. We found that the rate of non-native alleles in wild populations was age dependent, underpinning the importance of using age profiles in the design of genetic conservation studies.

4. Genetic refuge and direct translocation of wild trout resulted in a rapid and significant decrease in the percentages of non-native alleles. Moreover, the genetic refuge strategy resulted in a significant reduction in the number of pure non-native individuals, without changing trout densities, whilst direct translocations resulted in the establishment of dense, self-sustaining native trout populations. Direct translocations changed the distribution of genotype categories and increased densities up to 55-fold in 3 years. Our results also showed that angling resulted in a selective pressure on non-native trout introduced at fry stage, whereas non-native trout issued from natural recruitment were not affected.

5. Our study provides insights for improving the efficacy of practical conservation policies and can be used in other native freshwater fish conservation plans. Proactive measures such as direct translocation need to be implemented together with passive approaches such as genetic refuge policies. Before implementing such actions, accurate genetic and demographic studies at small geographical scales are essential to ensure that no self-sustaining population of non-native fish is present. To obtain rapid colonisation, we recommend introducing fish along whole river sections rather than concentrating on a few river stretches. Angling pressure can be used as an additional tool to improve restoration.

Keywords: brown trout, conservation in practice, genetic refuge, native salmonids, translocation

Introduction

Over the last two decades, the conservation of freshwater fish inter- and intra-specific diversity has become an important challenge for scientists and wildlife managers, especially concerning salmonids (Waples & Hendry, 2008). Brown trout (*Salmo trutta* L.) is an interesting model organism for freshwater fish conservation issues. The species displays a high level of ecological, genetic and phenotypic diversity (i.e. different morphologies, behaviours, life histories) throughout its native regions in Europe (Elliott, 1994; Bernatchez, 2001; Klemetsen *et al.*, 2003). Diversity is locally threatened by human activities (environmental changes, overfishing and invasion by non-native fish). Yet, it is also of considerable socio-economic value (Laikre, 1999). There is evidence that brown trout intra-specific diversity reflects adaptations to local environments (Hindar, Ryman & Utter, 1991; Ryman, 1991; Pakkasmaa & Piironen, 2001). Thus, in terms of conservation, maintaining the highest possible genetic integrity in local brown trout populations is crucial to ensure evolutionary processes and thereby the long-term conservation of the species (Taylor, 1991; Ryman, Utter & Laikre, 1995; Laikre, 1999; Utter, 2004).

In France, genetic studies of brown trout populations revealed the co-occurrence of two genetically distinct brown trout, that is, Atlantic trout and Mediterranean trout –named after their assumed origin according to the hydrographical drainage basin they inhabit (Guyomard, 1989; Bernatchez, Guyomard & Bonhomm, 1992; Estoup *et al.*, 2000). For more than a century, the Mediterranean catchment has been stocked with hatchery stocks of Atlantic origin (Krieg & Guyomard, 1985). Several studies have shown that Atlantic trout has replaced Mediterranean populations in many streams of the Mediterranean basin (Barbat-Leterrier, Guyomard & Krieg, 1989; Beaudou, Cattaneo-Berrebi & Berrebi, 1994; Poteaux, Beaudou & Berrebi, 1998; Caudron *et al.*, 2011a). In contrast, in some Mediterranean streams, despite similar stocking practices, native populations showing little genetic introgression by the Atlantic gene pool can still be found (Barbat-Leterrier *et al.*, 1989; Largiadèr & Scholl, 1996). Conservation of remnant native populations that display low introgression rates should be integrated into management programmes to protect the well-adapted local populations. Monitoring and assessment programmes will be important for detecting early effects on native populations as well as for refining restoration programmes.

In this study, we conducted a spatiotemporal genetic and demographic monitoring of a brown trout population subjected to two conservation strategies, that is, genetic

refuge and direct translocation of wild trout, programmes commonly used in the restoration of native salmonid populations (Maitland, 1995; Moritz, 1999; Schmetterling, 2003; Araguas *et al.*, 2009). Our aims were to assess the early effects and efficacy of these strategies on native Mediterranean brown trout populations in the Borne River, and, if necessary, to suggest modifications and improvements.

Methods

Study area and management actions

The Borne River is a fast-flowing mountain stream located in the Northern French Alps (length, 32 km; catchment area, 158 km²). It is a typical Alpine stream with a 3.3% mean slope and an altitude ranging from 2750 to 400 m a.s.l. The Borne River, situated in the Rhône drainage basin, lies within the geographical range of Mediterranean brown trout (Guyomard, 1989; Bernatchez *et al.*, 1992; Estoup *et al.*, 2000; Cortey, Pla & García-Marín, 2004). Mediterranean brown trout populations are therefore considered as the native species, while non-native Atlantic brown trout have been stocked intensively by fishery managers since 1913 (Caudron, Champigneulle & Guyomard, 2009b).

A previous study revealed a strong spatial structure, with a nearly pure native Mediterranean population downstream and a non-native Atlantic population upstream of a physical barrier that prevents migration upstream but allows migration downstream (Fig. 1) (Caudron *et al.*, 2009b). The native downstream population was characterised by a high frequency of Mediterranean alleles (90%) and a high population density (43–55 fish/100 m²). In contrast, the non-native upstream population was characterised by a high frequency of Atlantic lineage alleles (87%) and a low density (1–2 fish/100 m²) and appeared to be sustained by restocking (Caudron *et al.*, 2009b).

In the light of these results, a conservation and restoration programme was implemented over 6 years (2005–10) with different aims and actions in the downstream and upstream zones. In the downstream zone, the aim was to decrease the proportion of non-native Atlantic alleles in the remnant population to reduce its potential impact as a source of non-native alleles. Therefore, a genetic refuge strategy was implemented in 2005, and stocking of non-native Atlantic trout was terminated. Recreational angling was maintained because the abundant native population was not threatened and because angling seems to be selective, removing non-native

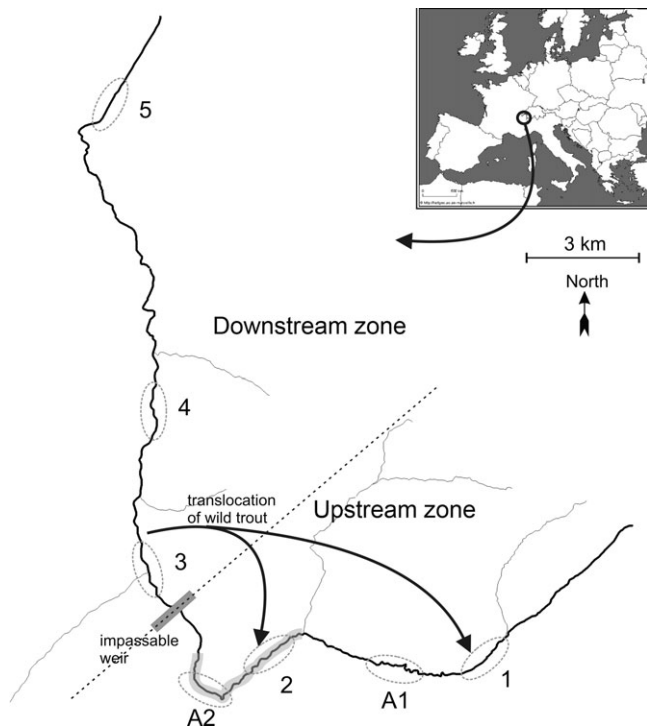


Fig. 1 Map of the study area showing the location of the study sites. The bold-type grey line represents the stretch of river where fishing was banned.

individuals before sexual maturity (García-Marín, Sanz & Pla, 1998; Mezzera & Largiadèr, 2001). In the upstream zone, inhabited by non-native Atlantic trout, the aim was to restore the native Mediterranean gene pool by establishing a new self-sustaining population of native brown trout. Therefore, direct wild trout translocations were carried out from the almost pure native Mediterranean population located downstream of the weir. Thus, for three consecutive years, 2005, 2006 and 2007, respectively, 260, 890 and 480 trout of various age classes were introduced into two sectors named sector 1 and sector 2 (Fig. 1): 135, 282 and 110 trout in sector 1 and 125, 610 and 370 trout in sector 2 for the 3 years, respectively. All the translocated trout were marked using adipose fin clipping to distinguish them from the wild population during monitoring. Fin clips were stored in 95% ethanol for genetic analyses. Recreational fishing was banned in a stretch of river including sectors 2 and A2 but not in the upper stretch including sectors 1 and A1 (Fig. 1).

Sampling and field investigations

In the downstream zone, in three sectors (3, 4 and 5), brown trout populations were monitored by electrofishing

before (2004) and after (2008) implementation of the genetic refuge strategy (Fig. 1). Trout density and biomass values were estimated using the two-catch removal method (De Lury, 1951). Each fish was anaesthetised, measured (to the nearest mm) and weighed (± 0.1 g). Scales and a fin clip were taken from trout chosen for genetic analysis and stored in 95% ethanol until analysed.

We studied the genetic characteristics of the trout caught by anglers for three age classes, that is, 1+, 2+ and 3+, to detect potential selective angling between wild and stocked origins. From 2002 to 2004, the otoliths of fry released into the Borne River at the yolk-sac fry stage were fluoro-stained with the fluorescent dye alizarin red S (Caudron *et al.*, 2009b). Hence, between 2004 and 2006, we were able to determine wild trout issued from natural recruitment from stocked fry in anglers' catches (legal size ≥ 23 cm). During this period, 258 trout, corresponding to the potentially fluoro-stained cohorts, were sampled by volunteer anglers and analysed. The age of each trout sampled in the monitored sectors and in anglers' catches was determined by scalimetry.

In the upstream zone, two sectors (1 and 2) where translocated trout was introduced were monitored before translocations in 2004 and after translocations in 2008, 2009 and 2010. In 2009, two additional sectors, A1 and A2 located two kilometres downstream of sectors 1 and 2, respectively, where no fish were directly introduced, were also studied to detect potential migrations of introduced trout or of their offspring (Fig. 1). Demographical monitoring, sampling and age determination of the trout studied in the upstream zone were carried out as in the downstream zone.

In addition, each caught trout was also checked for the presence of a potential adipose fin mark to identify specimens translocated between 2005 and 2007. Unmarked trout (i.e. with an adipose fin) had three different possible origins: non-native Atlantic trout still present in the zone, offspring of translocated native Mediterranean trout or offspring from a translocated native trout \times non-native trout mating. To determine the proportions of each origin, fin clips were taken for genetic analyses. These analyses enabled us to estimate the direct and indirect contributions of translocated trout to brown trout populations, and to determine whether translocations made it possible to establish a self-sustaining population.

Genetic analysis

Altogether, 876 trout were genotyped for two microsatellite markers, Str541 and Str591, located on two distinct linkage groups (BT2 and BT7, Gharbi *et al.*, 2006). These

microsatellite loci have been shown to differentiate between domesticated Atlantic stocks and unstocked Mediterranean trout populations over a large part of the Mediterranean area, including France, Italy, Greece and Spain (Estoup *et al.*, 2000; R. Guyomard, unpublished data). This method has been validated through genotyping at two adjacent markers (Str542 and Str592, respectively), which are physically closely linked to Str541 and Str591, respectively. In practice, the genotyping of the two additional loci Str542 and Str592 was restricted to a subsample of wild and fish-farmed individuals to determine the genetic origin (Mediterranean versus Atlantic) of the alleles observed at the Str541 and Str591 loci. The rationale of this approach is detailed in Estoup *et al.* (1999, 2000). DNA was extracted using Chelex resin following Estoup *et al.* (1996). Polymerase chain reaction amplifications were carried out following Launey *et al.* (2003).

Data analyses

Allele frequencies, average numbers of alleles and observed and expected unbiased 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 using Fisher's exact test in GENEPOP 3.4. These tests use a Markov chain method (1000 dememorisation steps, 100 batches and 1000 iterations per batch). To increase the power of the analysis, tests were performed after pooling Atlantic and Mediterranean alleles into two categories for each sample, following the approach of Mezzera & Largiadèr (2001).

Percentages of Atlantic alleles were estimated as the average frequencies of Atlantic alleles at the Str541 and Str591 loci (i.e. number of Atlantic alleles at Str541 and Str591 found in a sample divided by the total number of alleles observed in the same sample). The 95% confidence intervals of Atlantic allele frequencies were calculated based on Beyer's (1986) table, which is based on binomial distribution. Statistical differences in the proportions of Atlantic alleles between the different monitored periods were tested with a chi-square test. According to the observed numbers of hatchery-specific alleles at the two loci analysed (See Appendix S1), each individual analysed was assigned to one of the three following categories: pure hatchery trout, pure native trout and hybrid trout when 4, 0 and between 1 and 3 Atlantic alleles were found, respectively.

For populations sampled in the upstream zone in 2008 and 2009, a Bayesian method implemented in the programme STRUCTURE 2.2 (Pritchard, Stephens & Donnelly, 2000) was used to detect the potential presence of trout issued from transferred individuals from wild populations. In the case of introgression between native Mediterranean and non-native Atlantic origins, this software gives admixture rates very close to the introgression rates obtained by direct counting of Atlantic alleles at diagnostic markers (Caudron *et al.*, 2009a). This software can help to detect fish issued from wild population translocations even when there is no deviation from HW expectations (Caudron *et al.*, 2009a; Sanz *et al.*, 2009). To identify first-generation offspring of translocated individuals, individual admixture coefficients (q) were estimated for the 0+ and 1+ populations sampled after wild trout translocations in 2008 and 2009. For this analysis, the samples identified as non-native in 2004 ($n = 38$) and as native individuals translocated in 2005, 2006 and 2007 ($n = 123$) were used as non-admixed 'learning' samples, assuming an admixture model with two populations. A model of independent allele frequencies was assumed, and a burn-in period of 100 000 iterations was used, followed by 1 000 000 iterations. Further model parameters used for our analysis were GENBACK = 1 and MIGPRIOR = 0.00. The analysis was run five times to ensure result consistency.

Results

Direct translocations of trout to the upstream zone

In the 2004 samples, the multi-locus test performed with all the alleles and with alleles pooled into two categories (native Mediterranean versus non-native Atlantic) did not yield any significant departures from HWE for either sector 1 or 2, although a significant linkage disequilibrium was observed only for sector 2 (Table 1).

In sector 1, the tests showed significant departures from HWE and significant linkage disequilibrium only for the 2008 samples. In sector 2, no significant departures from HWE were detected, but the two loci were not in linkage equilibrium for the 2008 and 2009 samples. The additional samples taken in 2009 showed significant departure from HWE for sector A1, but no significant departure for sector A2. The two sectors showed significant linkage disequilibrium (Table 1).

In sector 1, no Mediterranean allele was found before translocations in the 2004 sample, whereas five Mediterranean alleles at Str591 (164, 166, 170, 188 and 192) were observed after translocations, in the 2008 and 2009

Table 1 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 together

	All-allele data		Pooled allele data	
	HWE	LD	HWE	LD
Upstream zone				
Sector 1				
2004	NS	NS	NS	NS
2008	**	**	**	**
2009	NS	NS	NS	NS
Sector A1				
2009	**	*	**	**
Sector 2				
2004	NS	**	NS	**
2008	NS	**	NS	**
2009	NS	*	NS	**
Sector A2				
2009	NS	**	NS	**
Translocated fish				
2005	NS	NS	NS	NS
2006	NS	NS	NS	NS
2007	NS	**	NS	**
Downstream zone				
Sector 3				
2004	**	**	**	**
2008	NS	NS	*	*
Sector 4				
2004	**	**	**	**
2008	NS	**	NS	**
Sector 5				
2004	NS	NS	NS	NS
2008	NS	**	NS	**
Angling catches				
Marked	NS	NS	NS	NS
Unmarked	**	**	**	**

* $P < 0.05$, ** $P < 0.01$.

samples. In sector 2, the presence of only two Mediterranean alleles at Str591 (188 and 192) was detected before translocations, whereas three additional Mediterranean alleles (Str591*164, 166 and 170) were observed after translocations. These new alleles correspond to the Mediterranean alleles at Str591 observed in translocated wild trout samples (Appendix S1).

For sectors 1 and 2, the frequencies of Atlantic alleles and the distribution of the three-two-locus genotypes (pure Mediterranean, pure non-native and hybrid) displayed a similar temporal pattern (Fig. 2). Between 2004 and 2008, the percentages of non-native alleles decreased significantly from 100 to 21% in sector 1 and from 91 to 17% in sector 2 ($P < 0.01$) and remained similar between 2008 and 2009. The distributions of three genotype categories were significantly different between 2004 and 2008, with a strong decrease in pure non-native genotypes, whereas the proportions of the pure native one and

of hybrid individuals increased. Genotype category distributions were similar between 2008 and 2009 (Fig. 2).

In sectors A1 and A2, sampled in 2009, the percentages of Atlantic alleles (34 and 69%, respectively) were significantly higher than in sectors 1 and 2 (17%) in the 2008 and 2009 samples, and percentages in sector A2 were significantly higher than those in sector A1. Genotype category distributions differed significantly between sectors A1 and 1, between sectors A2 and 2 and between sectors A1 and A2.

Individual admixture coefficients q , obtained for unmarked 0+ and 1+ trout, using STRUCTURE software, allowed us to establish the respective contributions of each geographical trout origin within the standing population (Fig. 3). Thus, trout that exhibited q values ranging from 0.75 to 0.9 with reasonable 90% probability intervals ranging from 0.2 to 1 were statistically assigned to first-generation offspring of translocated wild trout. In contrast, individuals exhibiting q values of 0.2 and lower, with 90% probability intervals ranging from 0 to 0.6, were statistically assigned to the non-native population. Finally, trout showing q values between 0.4 and 0.5, and 90% probability intervals not including values of 0 or 1 were likely to represent admixed individuals between native translocated trout and non-native trout still present in the upstream zone (Fig. 3).

The distributions of the different origins showed a similar pattern in sectors 1 and 2 in 2008 and 2009 (Fig. 3). Indeed, most trout, 86 and 89% in sector 1 and 78 and 83% in sector 2 in 2008 and 2009, respectively, were assigned to first-generation offspring of translocated wild trout. Less than 10% were statistically assigned to the non-native Atlantic population, and few (only 6–8% in sector 1 and 11–12% in sector 2) were considered as admixed individuals of Atlantic and Mediterranean origins. By contrast, the distributions of q values in sectors A1 and A2 in 2009 showed a different pattern (Fig. 3). In sector A1, 38% of trout were assigned to first-generation offspring of native translocated fish, and 62% represented admixed individuals between native and non-native trout. In sector A2, 61% of trout were assigned to the non-native population, 25% represented admixed trout, and only 14% were assigned to first-generation offspring of native translocated trout.

Density and biomass values were initially low in sectors 1 (2 individuals/100 m², 21 kg ha⁻¹) and 2 (1 individual/100 m², 11 kg ha⁻¹). In sector 1, trout densities were 6- to 20-fold higher after translocations, and biomass values were two-, four- and seven-fold higher in 2008, 2009 and 2010, respectively, than in 2004. After translocations, sector 2 displayed trout densities 20- to

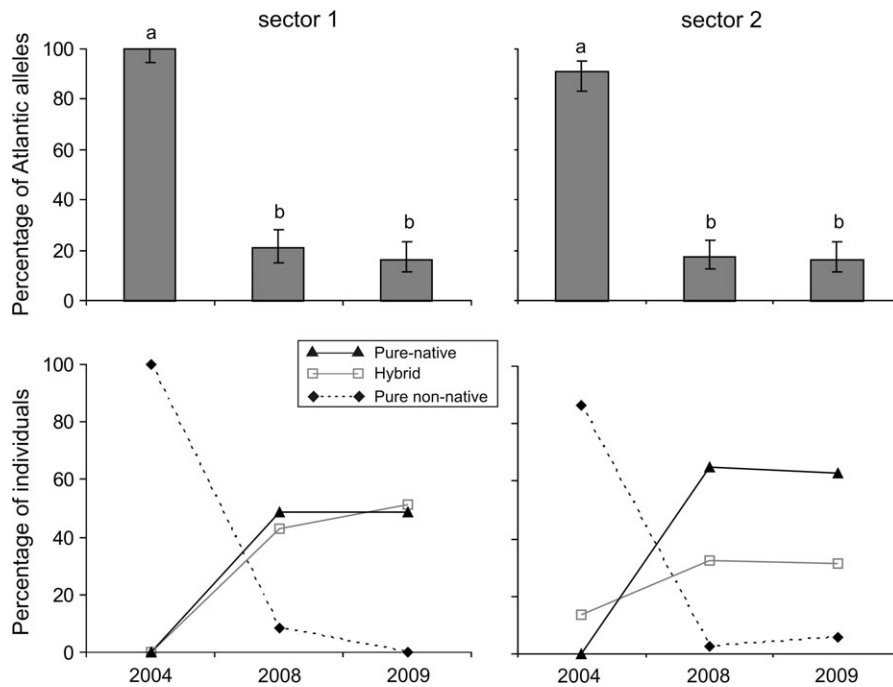


Fig. 2 Percentages of Atlantic alleles with 95% CI and percentages of native, hybrid and non-native individuals in two sectors studied before (2004) and after (2008, 2009) direct native trout translocations. Values with no letters in common are significantly different ($P < 0.01$).

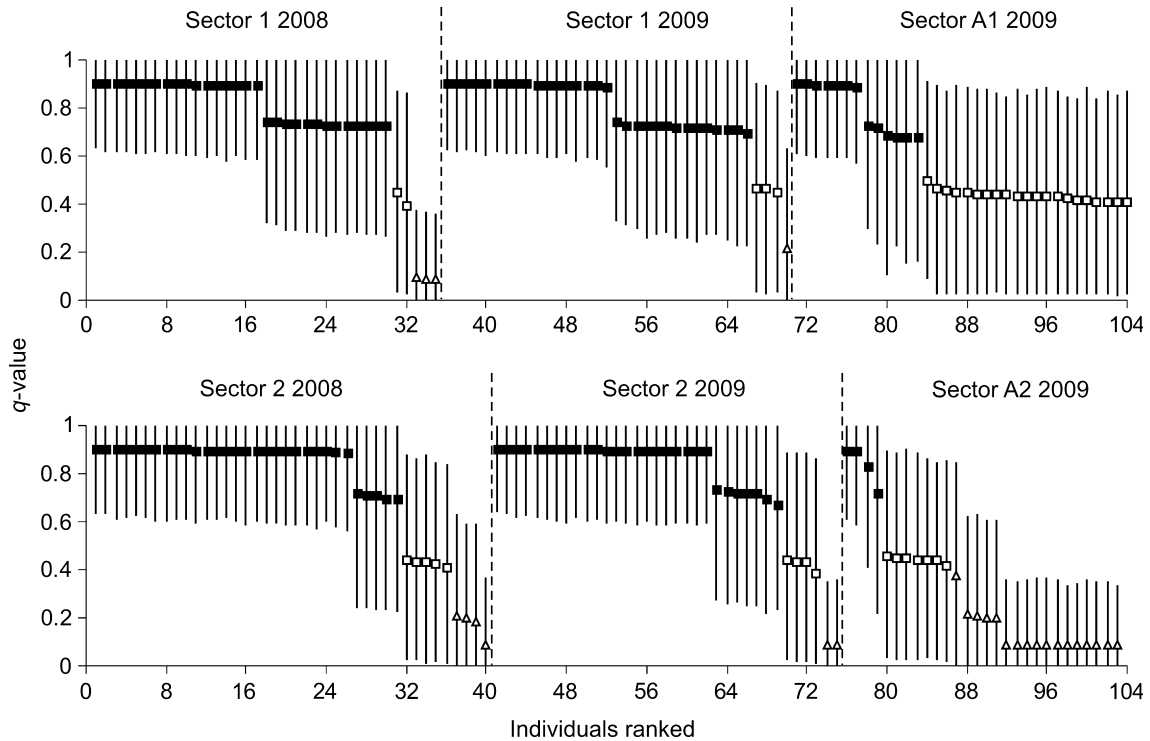


Fig. 3 Plots of individual admixture coefficients (q) in the upstream populations sampled after translocations in 2008 and 2009, estimated using STRUCTURE software and including 90% probability intervals. In this analysis, the number of populations was set to $K = 2$, and the samples collected prior to stocking were used as 'learning' samples (cf. Methods). A q value of one indicates trout issued from stocked Mediterranean fry, whereas a value of zero indicates pure non-native trout. Symbols indicate individual trout statistically assigned to the F1 offspring of translocated native population (■), to the non-native population (△) or to the admixed category (□).

Table 2 Brown trout density and biomass values obtained from maximum likelihood population estimation in the upstream sectors before translocations in 2004 and after translocations in 2008, 2009 and 2010

	Sector 1		Sector 2	
	Density (<i>n</i> /100 m ²)	Biomass (kg ha ⁻¹)	Density (<i>n</i> /100 m ²)	Biomass (kg ha ⁻¹)
Before translocation				
2004	2	21	1	11
After translocation				
2008	11	47	19	236
2009	41	78	53	241
2010	31	140	21	307

55-fold higher and biomass values 25- to 30-fold higher than in 2004 (Table 2). Density and biomass increases were higher in sector 2, where angling was banned, than in sector 1. Density and biomass values in 2009 in the sectors A1 (13.3 trout/100 m² and 54.3 kg ha⁻¹, respectively) and A2 (25.5 trout/100 m² and 126.6 kg ha⁻¹, respectively) were lower than in sectors 1 and 2. However, density and biomass values in sectors A1 and A2 after translocation were much higher than in sectors 1 and 2 before translocation. The relative contribution of marked trout (i.e. translocated trout) to wild population density and biomass values was lower for the 3 years (2008–10) after the last translocation (Fig. 4); no marked trout was caught in A1, and only 0.8% (3/356) were caught in A2.

In 2004, trout populations consisted of 8 and 12 individuals between 170 and 239 mm in sector 1 and in sector 2, respectively (Fig. 4). In contrast, after translocations, our results showed progressive wild trout establishment of various size classes with a decrease in the numbers of translocated trout (marked trout), and in particular large densities of young-of-the-year (<100 mm) (Fig. 4). In 2010, the population profiles observed in the upstream zone for wild unmarked trout differed strikingly from the profile observed in 2004 (Fig. 4) and showed similar profiles, density and biomass values to the wild population located in the downstream zone (data not shown).

Genetic refuge strategy in the downstream zone

Strong genetic effects were found in sectors 3 and 4 3 years after implementation of the genetic refuge strategy. Multi-locus tests using all alleles revealed significant departures from HWE for 2004, but not for the 2008 ($P > 0.05$) samples. Similar results were found when alleles were pooled into the two categories (native

Mediterranean versus non-native Atlantic) for sector 5. For sector 3, the two loci that showed significant linkage disequilibrium in the 2004 samples did not deviate significantly ($P > 0.05$) in the 2008 samples (Table 1).

Percentages of Atlantic alleles decreased significantly between 2004 and 2008 (30–6% in sector 3; 24–10% in sector 4) (Fig. 5). Distributions of genotype categories also differed significantly between 2004 and 2008 for sectors 3 and 4, with a strong decrease in pure non-native individuals in sector 3 and a disappearance in sector 4, whereas the rate of pure native individuals increased and the rate of genetically admixed trout (hybrid individuals) remained stable in the two sectors (Fig. 5).

Unlike sectors 3 and 4, sector 5 did not display any significant genetic difference between 2004 and 2008. Moreover, tests did not reveal any significant departures from HWE or any linkage disequilibrium, and the percentages of Atlantic alleles and the contributions of the different genotypic categories remained statistically similar in the standing populations during the 2004–08 period (Table 1; Fig. 5). This sector displayed a larger proportion of hybrid individuals (50%) in both 2004 and 2008 samples (Fig. 5). After 3 years without stocking, density and biomass values remained stable and high (40–50 individuals/100 m² and 200–250 kg ha⁻¹) between 2004 and 2008 for the three sectors in the downstream zone.

Samples from the three downstream sectors were pooled to compare the genetic characteristics of the 1+, 2+ and ≥3+ age classes. Between age classes differences were found in 2004 when sites were stocked with non-native trout, but no differences were noted in 2008, 3 years without stocking (Table 1; Fig. 6). Thus, in 2004, the multi-locus tests performed with all alleles and with the same alleles pooled into two categories (native Mediterranean versus non-native Atlantic) showed significant departures from HWE for 1+ and 2+ age classes, whereas the tests were not significant ($P > 0.05$) for the 3+ age class. The linkage equilibrium tests between the two loci gave similar results. Percentages of Atlantic alleles were significantly higher in the 1+ age class than in the 2+ and 3+ age classes and significantly higher in the 2+ age class than in the 3+ age class (Fig. 6). Distributions of the genotype categories showed significant differences between age classes, with a decrease in pure non-native genotypes between the 1+ and 2+ age classes and between the 2+ and ≥3+ age classes, while the rate of pure native genotypes increased. In contrast, the rate of hybrid trout remained statistically similar between the three age classes (Fig. 6).

Genetic analyses of fluoro-stained trout (i.e. stocked trout) in anglers' catches confirmed the non-native

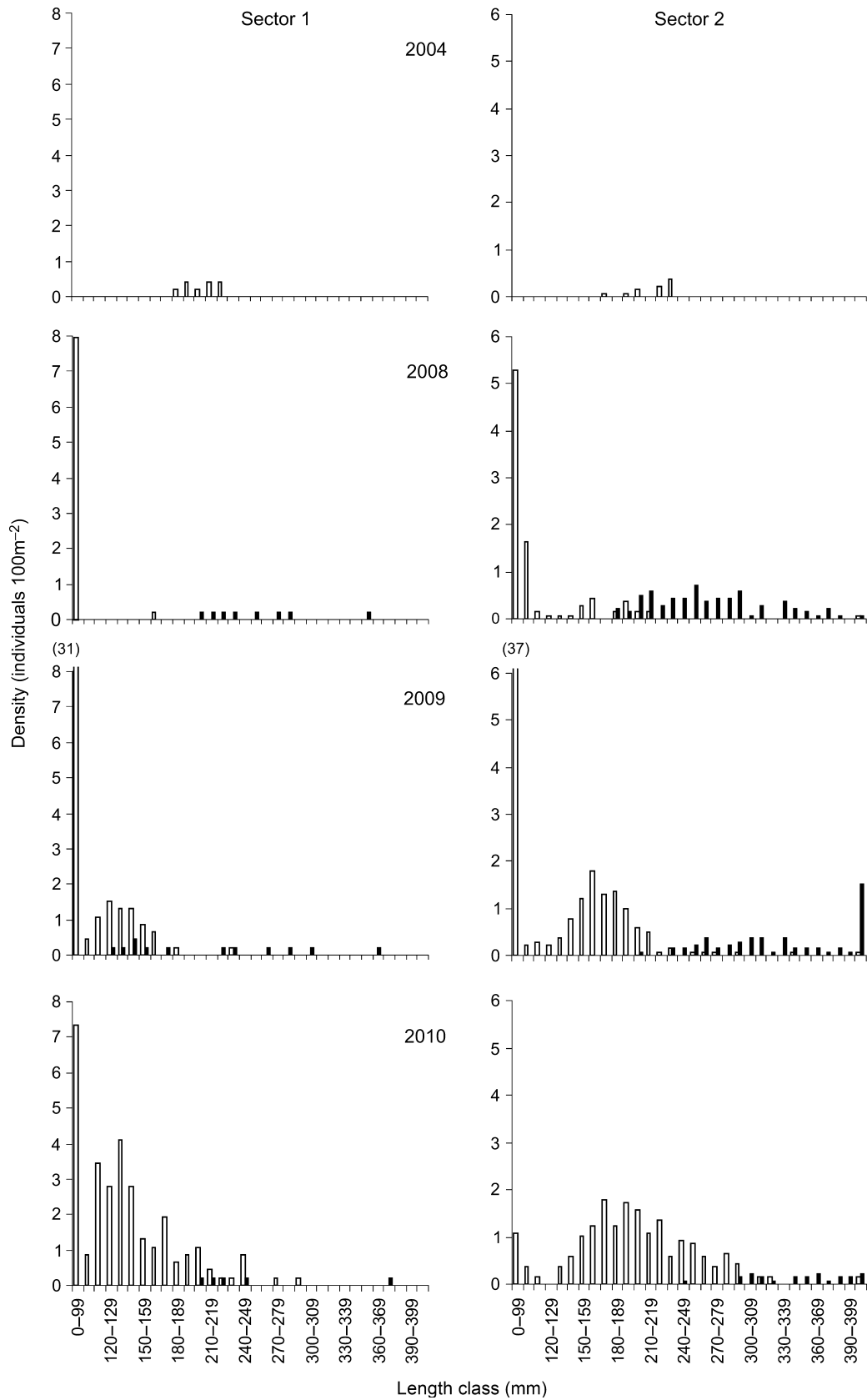


Fig. 4 Size distribution of brown trout densities in the upstream sectors (1 and 2) before (2004) and after (2008, 2009, 2010) translocations. Marked individuals (i.e. translocated fish) are represented in black, while unmarked fish (i.e. natural recruitment) are shown in white.

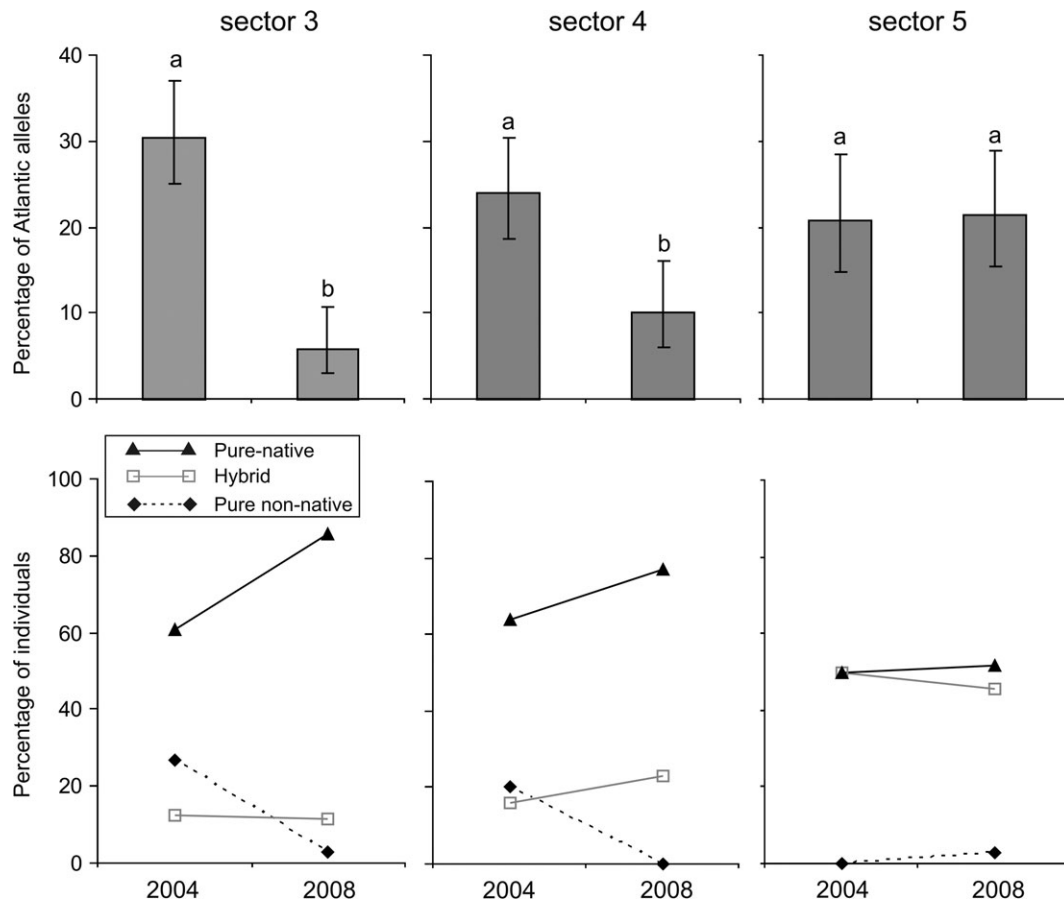


Fig. 5 Percentages of Atlantic alleles with 95% CI and percentages of native, hybrid and non-native individuals in three sectors studied before (2004) and after (2008) implementation of the genetic refuge policy. Values with no letters in common are significantly different ($P < 0.01$).

Atlantic origin of the trout used to stock the river (Appendix S1). Percentages of Atlantic alleles were significantly higher in total angling catches compared to the standing population sampled by electro-fishing in autumn for the 1+ ($P < 0.05$) and 2+ ($P < 0.01$) age classes, whereas no significant ($P > 0.05$) differences were observed between unstained catches and the standing population. The significant differences observed in the 1+ and 2+ age classes were no longer significant in the 3+ age class. The same pattern of differences between the three different samples was also observed for the distributions of genotype categories (Fig. 7).

Discussion

Our study showed the importance of collaboration between scientists and managers responsible for implementing and assessing the effects of a conservation programme. Recent studies have shown that scientific results are rarely converted into actions by resource managers (Pullin & Knight, 2001; Sutherland *et al.*, 2004;

Sarewitz & Pielke, 2007; Hart & Calhoun, 2010; Barmuta, Linke & Turak, 2011). Hence, development of collaborative research that merges scientific methods and management planning represents an effective process to bridge this gap (Caudron, Vigier & Champigneulle, 2012). Moreover, more applied studies documenting successful and unsuccessful conservation actions need to be published in the peer-reviewed literature (Pullin & Knight, 2009; Hulme, 2011).

The two strategies implemented here, genetic refuge and direct translocation of wild trout, resulted in fast and significant changes in the genetic and demographic characteristics of the standing brown trout populations. The results yielded four main conclusions discussed in following section.

The percentage of non-native alleles is age dependent

In the samples taken in 2004, which were directly influenced by stocking, the percentages of non-native alleles and the contribution of the three genotypes, native,

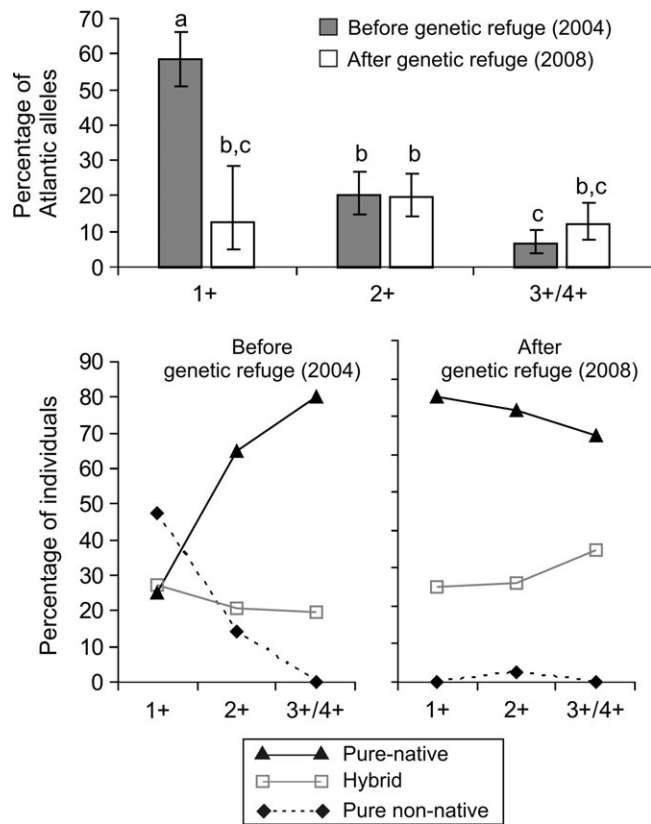


Fig. 6 Percentages of Atlantic alleles with 95% CI and percentages of native, hybrid and non-native individuals within the total sample of the downstream area for the three age classes (1+, 2+ and 3+/4+) before (2004) and after (2008) implementation of the genetic refuge policy.

hybrid and non-native, differed between age classes 1+, 2+ and 3+. This pattern can be explained by a decrease in the numbers of non-native genotypes stocked and disappearance of the 3+ and higher age classes. Our findings support those from earlier studies within the Rhône drainage basin (Mezzera & Largiadèr, 2001; Caudron *et al.*, 2011a,b). Moreover, a similar age-dependent percentage of non-native alleles is likely to occur in other brown trout lineages, for example, within the same lineage between domesticated and wild strains. Additional studies are therefore needed to better understand the effects of stocking, age-dependent migration of introgressed fish and a selection against non-native alleles and genotypes.

Information on the age profile of the fish populations was important for comparing the space- and time-dependent percentages of non-native alleles in standing populations and for interpreting the results. Indeed, the 'age profile' effect can cause biases in studies that assess the spreading of non-native alleles in stocked brown trout populations, confounding interpretation and ultimately

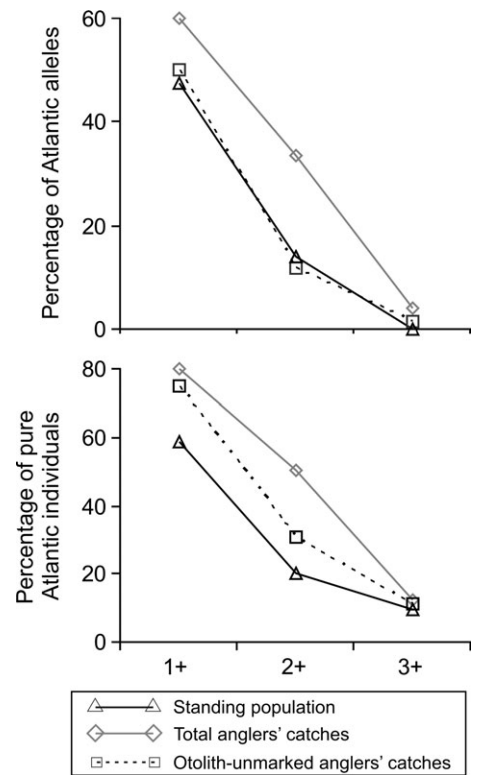


Fig. 7 Percentages of Atlantic alleles and percentage of pure Atlantic trout for the 1+, 2+ and 3+ age classes in three different samples: standing population, total catches (i.e. stocked trout and trout from natural recruitment) and otolith-unstained angler's catches (i.e. fish issued from natural recruitment only).

affecting conservation plans. However, few genetic studies indicate the age of the individuals analysed, even though samples are likely to be age-heterogenous. Therefore, we recommend that scalimetry analyses are used to obtain age profiles of the samples analysed and that comparisons are restricted to samples with similar age profiles or to individuals of the same age. When scalimetry analyses are not possible, we advise the use of a restricted length class range to avoid different ages. Second, we recommend that information of any recent stocking campaigns is obtained as the percentage of non-native alleles within samples can change rapidly (within 3 years in our study) after stocking has ceased.

Genetic refuges: a mitigated conservation strategy

Establishment of a genetic refuge can result in a fast and significant decrease in non-native Atlantic alleles. In our study, the observed decrease was mostly explained by the rapid disappearance of pure non-native individuals after stocking was terminated. However, the proportion of non-native alleles that introgressed into the native gene pool

did not decrease since the proportion of admixed individuals (hybrid category) remained stable in the three sectors studied. In sector 5, where no pure non-native trout were present in 2004, the rate of non-native alleles did not change between 2004 and 2008. Poteaux *et al.* (1998) showed that a genetic refuge of 5 years did not decrease the introgression of the Mediterranean native gene pool by the domesticated Atlantic strain in a southern French river. Likewise, Araguas *et al.* (2008, 2009) did not detect any genetic changes (rate of hatchery introgression, deviation from HWE or linkage disequilibrium) in Mediterranean populations of the eastern Pyrenees over a 13-year period after genetic refuges were established. According to these authors, the genetic refuge policy failed to erase the footprint of past stocking practices in the 10 rivers studied. Three other studies performed on streams in the Rhône drainage area, and using genetic refuge policies for 5 and 13 years, gave similar results (Caudron *et al.*, 2011a; Caudron & Champigneulle, 2011; C. R. Largiadèr, unpubl. data). Combined, these results suggest that genetic refuge policies (i.e. stopping the introductions of non-native fish into a hydrographic network inhabited by remnant native populations) yielded equivocal results. However, this kind of strategy is the first conservation measure that should be implemented by managers in areas harbouring nearly pure native populations, since it can reduce the potential source of further introgression. The efficiency of this passive approach could be improved by trying additional active measures, such as removal (e.g. selective angling) of non-native fish, stocking with local native breeding stock and/or translocating wild individuals, and by assessing their efficiency for restoring the genetic integrity of native populations.

Direct translocations as an efficient restoration strategy, but with local effects only

Translocating native trout, together with the termination non-native trout stocking, resulted in a rapid decrease in the percentages of non-native alleles and a shift in the distribution of genotype categories. Our results show that this strategy made it possible for high-density, self-sustaining populations to develop within 3 years. Trout densities increased 20- to 55-fold after translocations, and most (78–89%) juvenile trout were first-generation translocated wild trout found in the sectors where they were introduced. However, genetic and demographic effects appear restricted to the river stretches where translocated trout were introduced. Indeed, 2 km downstream of the sectors where trout were introduced, the percentages of

non-native alleles remained significantly higher, trout densities were lower than in the upper sectors directly influenced by translocations, and the proportions of first-generation offspring assigned to native translocated fish were lower. Therefore, we recommend that fish are introduced along large areas of the river rather than restricted to a few river stretches. It is essential to transfer a sufficient number of individuals belonging to a wide age class range (Minckley, 1995; Fisher & Lindenmayer, 2000), in order to reflect the genetic composition of the source population (Stockwell, Mulvey & Vinyard, 1996), and to occupy at least 10% of the carrying capacity of the receiving site (Hilderbrand, 2002). Furthermore, the translocation of native fish in parts of rivers inhabited by non-native fish can lead to admixed fish which can persist over time. Therefore, before implementing direct native fish translocations, an accurate genetic and demographic analysis should be carried out to ensure that no self-sustaining populations of non-native fish are present.

Angling as an additional conservation action

Studies suggest that domesticated Atlantic brown trout are more likely to be caught by anglers than native Mediterranean brown trout (García-Marín *et al.*, 1998; Mezzera & Largiadèr, 2001; Champigneulle & Cachera, 2003). Our study showed that the proportion of pure Atlantic trout was significantly higher in total anglers' catches than in the standing population and that genetic differences between the naturally recruited trout found in catches and the standing population were not significant. This finding indicates that stocked fish are preferentially caught and suggests that fishing would have little selective effects on natural populations, at least with regard to the Atlantic/Mediterranean origin of alleles. However, this conclusion needs to be confirmed with the use of additional diagnostic markers.

According to Arlinghaus (2006) and Granek *et al.* (2008), anglers are not necessarily a threat to native populations and can act as conservation partners in some instances. Thus, when the demography of a native population is high enough to allow angling activity, selective angling pressure on non-native stocked trout can function as a complementary tool to limit the potential risk of further introgression in the first years after the genetic refuge area has been established. According to our results, this tool was effective for removing non-native stocked trout but was not effective for removing trout from a non-native, self-sustained population. However, several authors have shown angling to have a limited impact on non-native populations. Larson, Moore & Lee (1986) showed that

angling pressure reduced the non-native rainbow trout (*Oncorhynchus mykiss* Walbaum) population in an Appalachian stream by only about 10%. Other studies, carried out in Rocky Mountains creeks in Alberta (Paul, Post & Stelfox, 2003), found that non-native brook trout (*Salvelinus fontinalis* Mitchell) populations are highly resilient to overexploitation; therefore, selective angling exploitation was ineffective at eliminating non-native trout populations. Our results showed that improvement was faster in the river stretch where angling had been banned than in the sections where angling was permitted. However, the self-sustained populations obtained 3 years after translocations displayed higher Mediterranean allele frequencies in the fishing zone than in the no-fishing zone. Hence, fishing pressure could be used as an additional tool to improve future restoration plans, but there needs to be a balance between selective angling of non-native fish and overfishing that might compromise restoration efforts.

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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 Str541 and Str591 diagnostic loci for sample analysed.

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