

# Stocking of native Mediterranean brown trout (*Salmo trutta*) into French tributaries of Lake Geneva does not contribute to lake-migratory spawners

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**Abstract** – We studied the contribution of stocking with Mediterranean autochthonous brown trout (*Salmo trutta*) to the lake-migratory spawners in French tributaries of Lake Geneva. This singular lake system is inhabited by both the resident and the migratory life-history form, and by trout of Atlantic and Mediterranean origins. Using two discriminatory microsatellite loci between the Atlantic and Mediterranean alleles, we compared the genetic composition of the migratory Atlantic populations of the four main tributaries before (1999;  $N = 113$ ) and after (2004, 2005 and 2006;  $N = 137$ ) recent introductions of native Mediterranean trout. No significant modification of the genetic composition was detected in all four populations over time and only two individuals were statistically assigned to the Mediterranean strain by the Bayesian method implemented in the STRUCTURE program. These results suggest a negligible contribution of the stockings with autochthonous Mediterranean trout to the lake-migratory spawners migrating into the four tributaries.

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**Key words:** lake-migratory trout; Mediterranean origin; microsatellite; stocking contribution

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## Introduction

The brown trout (*Salmo trutta* L.) shows a huge ecological plasticity and a high genetic diversity. Bernatchez (2001) identified five major evolutionary mtDNA lineages: Atlantic, Mediterranean, Marmoratus, Adriatic and Danubian. Ecologically, two main life-history forms, resident and migratory (the sea trout or the lake-migratory trout) can coexist in the same basin and within the same population (Jonsson & Jonsson 1993; Ferguson 2006). To conserve the genetic diversity of the brown trout, it is essential to take into account this intra-specific variability (Ferguson 1989; Laikre 1999). This applies, in particular to systems where different components of the intra-specific trout diversity are present in sympatry.

The Lake Geneva drainage system is inhabited by both the resident and the migratory life-history forms

of *S. trutta* (Champigneulle et al. 1999), and by trout of Atlantic and Mediterranean origins, respectively. Previous studies showed in the Lake Geneva system that (i) the migratory populations in most tributaries display frequencies of more than 90% of Atlantic alleles (Largiadèr et al. 1996; Launey et al. 2003); (ii) a native Mediterranean resident population is present in the upper part of the Dranse River (Guyomard 1989; Largiadèr et al. 1996) and (iii) the migratory population shows significantly higher frequencies of Mediterranean alleles in the Dranse River than in the other tributaries (Launey et al. 2003). The origin of the Atlantic trout in this area is not clarified yet and two nonexclusive hypotheses have been proposed (Largiadèr et al. 1996): a natural colonisation through a postglacial corridor between both Rhine and Rhône basin and an anthropogenic origin due to stocking with trout of Atlantic origin. The first hypothesis is supported

by the presence in the Lake Geneva basin of autochthonous whitefish (*Coregonus lavaretus*), Arctic charr (*Salvelinus alpinus*) and bullhead (*Cottus gobio* L.), which have colonised the basin from the North (Brunner et al. 1998; Vonlanthen et al. 2007). In favour of the stocking hypothesis, the Lake Geneva basin has been stocked intensively for about one century with domesticated trout of Atlantic origin (Largiadèr et al. 1996; Champigneulle et al. 1999; Rubin 1999). The sustainable management of brown trout populations in such a particular hydrogeographic network is thus a challenging task as it must take into account two ecological forms and two origins.

Until 1999, all French tributaries of Lake Geneva have been stocked only with domesticated Atlantic trout. Since 2000, the French managers changed their stocking practices and trout of two different origins have been used to stock these tributaries: an Atlantic domestic strain and a Mediterranean captive broodstock. This stock was founded using autochthonous Mediterranean resident spawners located in the upper Dranse River. The French fisheries managers mainly aimed at increasing the Mediterranean autochthonous resident population by stocking the offspring of this local strain. In the Dranse River system, a mass marking experiment showed that the stocked Mediterranean trout accounted for 10–30% of the resident trout caught by anglers (Caudron & Champigneulle 2007). In the Ugine River, an upstream tributary of the Dranse River, stocking with Mediterranean trout resulted in a natural recruitment of Mediterranean fry and a high increase of the Mediterranean allele frequencies (Caudron et al. 2006).

The present study attempts to assess whether the Mediterranean trout of the cohorts, which have been stocked in the French tributaries of Lake Geneva, contributed significantly to the spawning stocks of lake-migratory trout in these tributaries. To this aim, a genetic temporal analysis, before and after the stocking with Mediterranean fish was carried out to detect the potential contribution of the Mediterranean fry stocked to the migratory populations of the four major French tributaries of Lake Geneva.

## Materials and methods

### Location of the investigated sites

The migrant spawners were sampled in the downstream parts of the four main French tributaries of Lake Geneva where stocking with Mediterranean trout has been carried out: Pamphiot, Redon, Foron and the lower Dranse (Fig. 1). The access of the tributaries by migratory trout spawners is restricted to their downstream part due to the presence of impassable obstacles (Fig. 1).

### Restocking practices

All the French tributaries of Lake Geneva had been intensively stocked with hatchery stocks of Atlantic origin since about one century until 1999. Since 2000, the stocking practices have been changed and both Mediterranean and Atlantic strains have been used. The captive Mediterranean breeding stock has been founded with wild autochthonous Mediterranean resident spawners caught in the Dranse d'Abondance River (Fig. 1). In this section of the river, the introgression rate of Atlantic alleles is <5% (Caudron 2008).

The quantities and origins (Atlantic and Mediterranean) of fry stocked in each studied stream and for each of the following cohorts (1995, 1996, 1997 and 2000, 2001, and 2002) are reported in Table 1. For the period 2000–2002, Atlantic trout were stocked only in the lower part of the rivers. All the Atlantic and Mediterranean trout were released as fed fry.

### Field sampling

In each tributary, the lake-migratory spawners belonging to the cohorts born in 1995, 1996 and 1997, were sampled in 1999 before stocking with Mediterranean fish occurred. Fish belonging to the 2000, 2001 and 2002 cohorts, which were potentially under the influence of the stocking with Mediterranean trout, were sampled in 2005 and 2006.

In the Pamphiot, Redon and Foron electro-fishing was performed during the spawning period in 1999/2000 (1995, 1996 and 1997 cohorts), prior to stocking with Mediterranean fish and in winter 2004/2005 (2000, 2001 and 2002 cohorts), after the Mediterranean stocking has started. For those three streams, the sampling extended across the whole downstream part colonised by the lake-migratory trout spawners.

The strong flow in the lower part of the Dranse River made sampling by electro-fishing unrealisable in this river. Thus, fish were trapped in a fish-pass located downstream, at Vongy (Fig. 1). All fish were collected during the main autumn–winter spawning-migration period in 1999 (1995, 1996 and 1997 cohorts) and in 2005/2006 for the second sampling (2000, 2001 and 2002 cohorts).

In the case of these tributaries, the lake-migratory trout spawners were easily distinguished from sedentary trout in the field by their large size (due to their rapid growth in lake) and their silvery body colour (Jonsson & Jonsson 1993; Baglinière et al. 2001). Nevertheless, to increase the probability to include only migratory individuals into the analyses, scale samples of each fish were analysed in order confirm the presence of a rapid growth pattern, which is

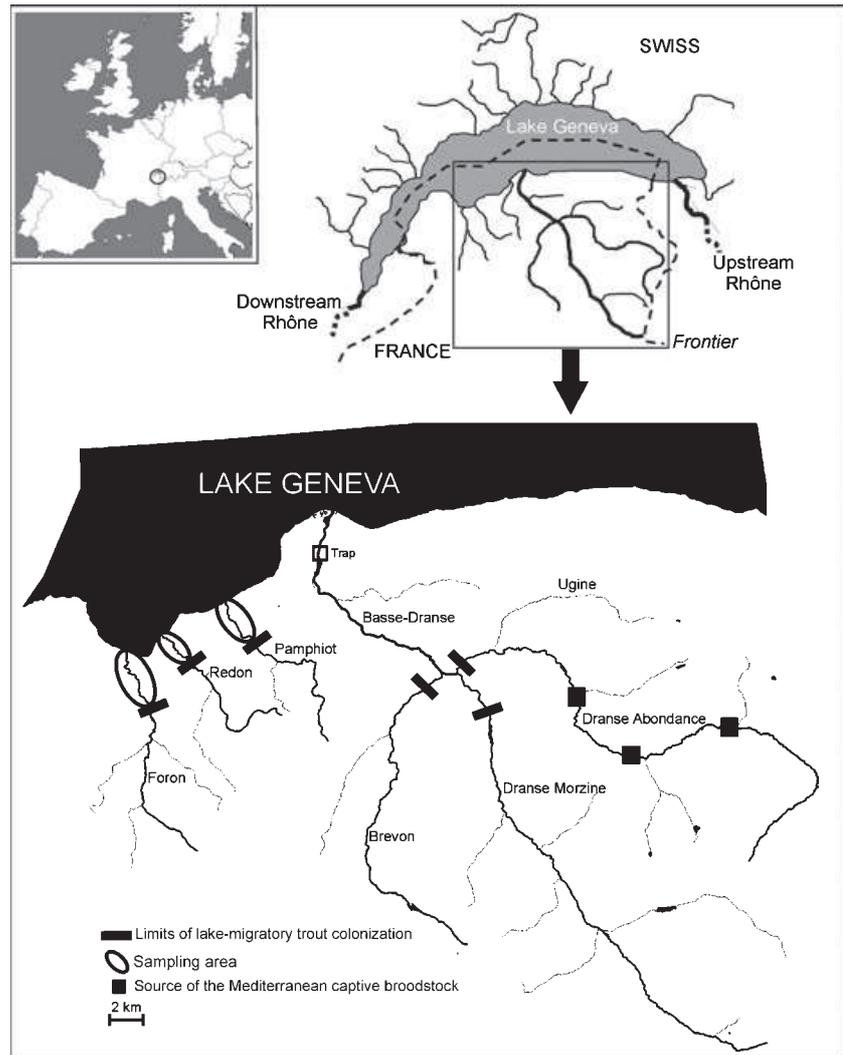


Fig. 1. Sampling locations in the four French tributaries studied that are located in the Lake Geneva drainage system. In the Pamphiot, Redon and Foron, the fish were caught with electro-fishing and in the Dranse the trout were sampled using a trap installed in a fish-pass (located on the map).

Table 1. Quantity and origin of fry stocked in the four investigated stream for each cohort studied.

Origin	Year/cohort	Dranses	Pamphiot	Redon	Foron
Atlantic	1995	722,000	42,500	76,000	80,000
	1996	759,000	44,000	76,000	80,000
	1997	674,000	47,000	80,000	90,000
	2000	21,000	1250	1500	1500
	2001	28,000	3000	3000	4000
	2002	13,600	2000	1500	2500
Mediterranean	1995	0	0	0	0
	1996	0	0	0	0
	1997	0	0	0	0
	2000	157,200	14,600	26,300	27,200
	2001	225,600	19,000	30,000	34,000
	2002	181,500	17,000	21,700	27,300

characteristic of migratory trout (Baglinière et al. 2001).

For each lake-migratory trout spawner 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. The number of trout analysed per sampling site and cohort is reported in Table 2.

### 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 showed 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 geographic 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

Table 2. Quantity of trout analysed in each studied site and for each cohort during both periods affected by stocking with Atlantic and Mediterranean trout.

Year of sampling	River	Cohorts affected by Atlantic stocking				Cohorts affected by Mediterranean stocking			
		1995	1996	1997	Total	2000	2001	2002	Total
1999	Foron	2 (4+)	21 (3+)	6 (2+)	29	–	–	–	–
	Pamphiot	0	8 (3+)	11 (2+)	19	–	–	–	–
	Redon	1 (4+)	2 (3+)	32 (2+)	35	–	–	–	–
	Basse-Dranse	24 (4+)	5 (3+)	1 (2+)	30	–	–	–	–
	Total	27 (4+)	36 (3+)	50 (2+)	113	–	–	–	–
2004	Foron	–	–	–	–	10 (4+)	17 (3+)	16 (2+)	43
	Pamphiot	–	–	–	–	9 (4+)	11 (3+)	6 (2+)	26
	Redon	–	–	–	–	6 (4+)	8 (3+)	17 (2+)	31
2005–2006	Basse-Dranse	–	–	–	–	0	11 (4+, 5+)	26 (3+, 4+)	37
	Total	–	–	–	–	25	47	65	137

Estoup et al. (1999, 2000). The results on the samples of 1999 derived from Launey et al. (2003). The alleles frequencies found in the ML strain used for stocking the French tributaries have already been published in Caudron et al. (2006).

DNA was extracted from the tissues of the other samples 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  $\mu$ l reaction volume containing 5  $\mu$ l Multiplex PCR Master Mix, 3  $\mu$ l dH<sub>2</sub>O, 1  $\mu$ l DNA and 1  $\mu$ l primer mix (2 pmol· $\mu$ l 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  $\mu$ 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). Genotypes were determined with the Genemap-3.0 (ABI) software and checked by eye.

#### Statistical analyses

The Atlantic allele frequencies were estimated as the average frequencies of Atlantic alleles (Table 1) 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). 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. Significant differences in allelic frequencies were tested with GENEPOP 3.4. These three tests used a Markov chain method (1000 dememorisation steps, 100 batches and 1000 iterations/batch). To detect recent effects of ML stocking in the samples and 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).

A Bayesian method implemented in the program STRUCTURE version 2.2 (Pritchard et al. 2000) was used to infer the number of populations ( $K$ ) independently of sampling sites and to detect the potential presence of stocked individuals in the wild populations. This approach is complementary to the use of discriminatory markers between Atlantic and Mediterranean origins in particular to detect specimen stocked in wild population even if HWE suggest a panmictic situation (Sanz et al. 2009). Firstly, to determine the number of populations, we used the admixture model with correlated allele frequencies without using any prior population information. Data were obtained with runs of 1,000,000 iterations (for  $K = 1 \dots 5$  and five replicates) and a burn-in of 100,000 iterations.

Secondly, to identify stocked individuals or F1-offspring of stocked individuals, individual admixture coefficients ( $q$ ) were estimated for all populations after the stocking with 0+ year old fish of the Mediterranean strain. For this analysis, the samples of 1999 of each population and of the Mediterranean strain were used as nonadmixed 'learning' samples. 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 in this analysis were: GENBACK = 1 and MIGPRIOR = 0.00. The analysis was run five times to verify the consistency of the results.

## Stocking contribution to lake-migratory trout

Table 3. Allele frequencies observed at the Str541 and Str591 loci for each sample before (1999) and after (2004 and 2005–2006) the Mediterranean stocking and for the fry used to stock the tributaries.

Year ( <i>n</i> )	Foron		Pamphiot		Redon		Basse-Dranse		Fry stocked
	1999 (29)	2004 (43)	1999 (19)	2004 (26)	1999 (35)	2004 (31)	1999 (39)	2005–2006 (50)	2000–2003 (77)
Str541									
<b>130</b>	<b>0.0345</b>	<b>0.0465</b>	<b>0.1053</b>	<b>0.0769</b>	<b>0.0429</b>	<b>0.0645</b>	<b>0.0256</b>	<b>0.0200</b>	<b>0.0000</b>
<b>132</b>	<b>0.8103</b>	<b>0.8372</b>	<b>0.8947</b>	<b>0.8269</b>	<b>0.9286</b>	<b>0.9032</b>	<b>0.7692</b>	<b>0.7900</b>	<b>0.0260</b>
136	0.1552	0.1163	0.0000	0.0962	0.0286	0.0323	0.2051	0.1900	0.9740
H n.b.	0.3237	0.2867	0.1935	0.3069	0.1371	0.1819	0.3703	0.3428	0.0509
H obs.	0.3793	0.3023	0.2105	0.2692	0.1429	0.1935	0.4615	0.3400	0.0519
Str591									
<b>150</b>	<b>0.8621</b>	<b>0.7442</b>	<b>0.7105</b>	<b>0.9038</b>	<b>0.8143</b>	<b>0.8065</b>	<b>0.8333</b>	<b>0.8100</b>	<b>0.0844</b>
<b>152</b>	<b>0.1207</b>	<b>0.2326</b>	<b>0.2368</b>	<b>0.0962</b>	<b>0.1429</b>	<b>0.1452</b>	<b>0.1026</b>	<b>0.0900</b>	<b>0.0000</b>
166	0.0172	0.0000	0.0263	0.0000	0.0143	0.0000	0.0128	0.0100	0.0714
170	0.0000	0.0116	0.0263	0.0000	0.0143	0.0323	0.0128	0.0000	0.1558
176	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0130
186	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0260
188	0.0000	0.0000	0.0000	0.0000	0.0000	0.0161	0.0000	0.0000	0.0000
190	0.0000	0.0000	0.0000	0.0000	0.0143	0.0000	0.0000	0.0000	0.0260
192	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0974
194	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0100	0.3571
196	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0128	0.0200	0.0000
198	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0128	0.0300	0.0844
200	0.0000	0.0116	0.0000	0.0000	0.0000	0.0000	0.0128	0.0300	0.0844
H n.b.	0.2462	0.3964	0.4495	0.1772	0.3205	0.3326	0.2980	0.3368	0.8160
H obs.	0.2759	0.4884	0.4737	0.1923	0.2857	0.2581	0.3333	0.2800	0.7922
<i>N</i> allele	3	3.5	3	2.5	4	3.5	5	5	6
Rate of Atlantic alleles	91%	93%	97%	94%	96%	96%	86%	85%	5%

*n* = sample size; H n.b. = expected unbiased heterozygote; H obs. = observed heterozygote; *N* allele = mean number of allele per population. In bold, the Atlantic alleles.

## Results

As expected, the large allele frequency differences were found between the wild stocks from the four tributaries and the Dranse captive population used for stocking. According to Estoup et al. (2000), alleles 130 and 132 for *Str541* and alleles 150 and 152 for *Str591*, respectively, were considered as Atlantic alleles and all others alleles as Mediterranean (Table 3).

The multi-locus tests performed with all the alleles and with alleles pooled in two categories (Mediterranean vs. Atlantic) for each period and for each sample did not reveal any significant (Fisher exact test,  $P > 0.05$ ) departures from HWE. The two loci were in linkage equilibrium.

The genetic differentiation tests showed that, for each locus and for the multi-locus test, frequencies did not differ significantly (Fisher exact test,  $P > 0.05$ ) between both periods (i.e. before and after Mediterranean stocking) for each of the four sampled tributaries (Fig. 2). Also, no significant differences (Fisher exact test,  $P > 0.05$ ) were observed when the alleles were pooled in two categories according to their Mediterranean or Atlantic origin. The percentage of Atlantic alleles remained high (between 85% and 98%) in the populations sampled before and after the

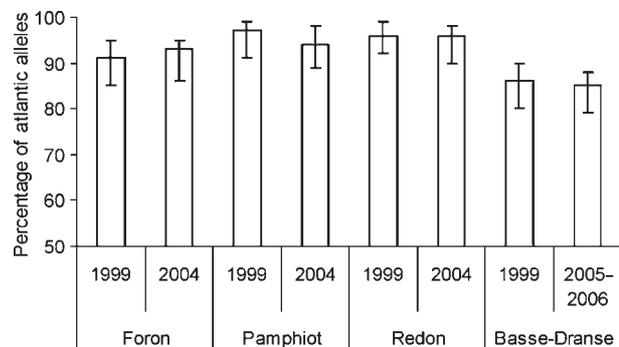


Fig. 2. Frequencies of Atlantic alleles at locus Str541 and locus Str591, respectively, in samples collected in the four tributaries studied before (i.e. in 1999) and after the stocking with Mediterranean trout (i.e. in 2004 and 2005–2006). The bars indicate the 95% confidence intervals of the estimated allele frequencies.

beginning of stocking with the Mediterranean strain. However, the Mediterranean allele frequency was significantly higher in the Basse-Dranse population than in the three other populations over both time periods (chi-square test,  $P < 0.01$ ).

In addition, the three most common Mediterranean alleles, 194, 170 and 192, observed at *Str591* in the captive broodstock with frequencies of 35.7%, 15.6% and 9.7%, respectively, were absent or nearly absent in

the four rivers after stocking with the Mediterranean strain (Table 3).

The STRUCTURE analyses revealed a clear signal of only two gene pools ( $K = 2$ ). In more detail, the probability estimates [ $\ln\text{Pr}(X|K)$ ] averaged across five replicate runs increased from  $-1466$  (with  $K = 1$ ;  $\text{SD} = 0.09$ ) to the maximum value of  $-1027$  (with  $K = 2$ ;  $\text{SD} = 0.71$ ) and then decreased from  $-1043$  (with  $K = 3$ ;  $\text{SD} = 2.17$ ) to  $-1103$  (with  $K = 5$ ;  $\text{SD} = 1.62$ ). It is worth mentioning that when the reference sample of the stocked Mediterranean fry was excluded from the analysis, the most likely  $K$  suggested by STRUCTURE was one, indicating that all eight samples from the wild populations were genetically highly similar (data not shown). All reported individual admixture coefficients ( $q$ -values) are thus based on a two-population model ( $K = 2$ ) using the samples of 1999 of each population and of the Mediterranean strain as nonadmixed ‘learning’ samples.

Only two fish of the Basse-Dranse 2005–2006 sample showed 90% probability intervals of the  $q$ -values not including a value of zero (Fig. 3). As a  $q$ -value of zero denotes a pure wild trout, only these two fish can be rejected from having a pure wild trout ancestry under the given model assumptions. Given the age of the two specimens (3+ years), they might represent stocked individuals or F1-offspring of a stocked individual and a wild trout.

## Discussion

Despite several years of stocking in the four main French tributaries of Lake Geneva, no evidence of a significant contribution of stocked Mediterranean trout to the lake-migratory spawner returns was detected. Sampling bias is unlikely to explain this finding as the analysed individuals represent a significant part, of the migrant spawners entering these French tributaries (at least 30%; Champigneulle et al. 2003 and Champigneulle, unpublished data). Moreover, for each period (before and after stocking with Mediterranean individuals), the three main age-classes (2+, 3+ and 4+ years) of migrant spawners have been sampled.

The interpretation that the released juveniles of the Mediterranean captive strain did not significantly contribute to migrant spawners entering the four tributaries during the main ‘autumn–early winter’ migration run is strongly supported by the following results:

(A) There was no general increase in Mediterranean allele frequencies in the lake-migratory trout spawners and the most frequent alleles (e.g. 170, 192 and 194 at the locus *Str591*) in the Mediterranean broodstock were not detected at higher frequencies than rare Mediterranean alleles. For example, the most frequent allele of the broodstock, *Str591*\*194 (frequency of

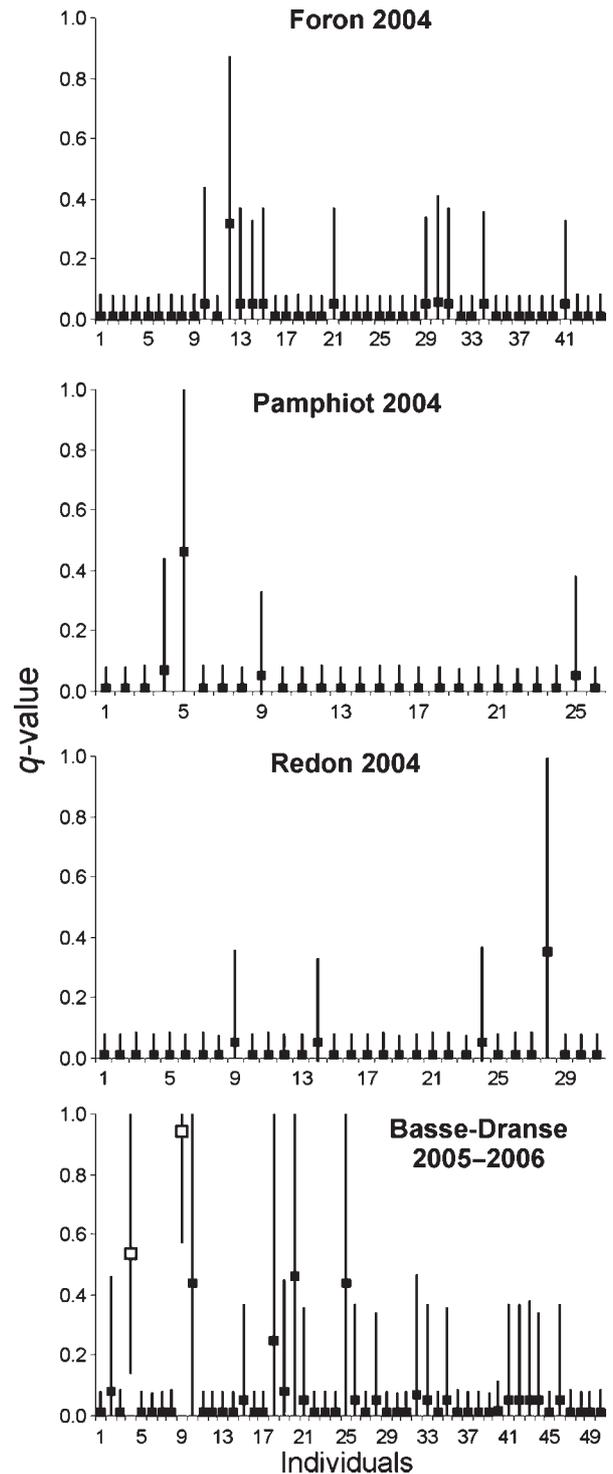


Fig. 3. Plots of individual admixture coefficient ( $q$ ) in the four populations sampled after stocking estimated by STRUCTURE including their 90% probability intervals. In this analysis, the number of populations was set to  $K = 2$  and the samples collected prior to the stocking were used as ‘learning’ samples (cf. Materials and methods section). A  $q$ -value of one denotes a trout derived from stocked Mediterranean fry, whereas a value of zero denotes a pure wild trout. The symbol  $\square$  indicates individuals having probability intervals of the estimated membership in the stocked Mediterranean strain that excludes a value of zero.

36.7%) was only observed once in 137 lake-migratory spawners. Assuming a genetic contribution of 5% of the Mediterranean broodstock to the spawners in the four populations should have been detected more than once with a probability of 95.7%.

(B) No significant departure from HWE nor from linkage equilibrium was found even when alleles were pooled according to their origin (Mediterranean or Atlantic). In the case of a recent admixture between Mediterranean broodstock and Atlantic trout populations, significant departure from HWE and linkage disequilibrium would be expected as the two strains were highly differentiated.

(C) According to the STRUCTURE analyses, of all 137 spawners that were sampled after stocking with Mediterranean trout, only two individuals (<1.5%), which had been caught in the lower Dranse, were statistically assigned to the Mediterranean strain. However, these two trout, might also have immigrated from the wild Mediterranean trout population from the upper part of the Dranse, where spawners have been caught to found the captive Mediterranean broodstock.

The general finding that the Mediterranean stockings did not contribute to lake-migratory trout spawners is in contrast with the results of a previous stocking experiment using trout of Atlantic origin in the Redon. In this tributary stocked with 6000–8000 alevins of Atlantic origin per year, at least 12–25 of the introduced trout returned to the Redon River as lake-migratory spawners (Champigneulle et al. 2003).

In the studied tributaries, with the exception of the Dranse, we found very few adult resident trout and so very few Mediterranean spawners in the downstream part colonized by the migrant spawners. It could be hypothesised that the Mediterranean broodstock used would be more adapted to residency in the upper high-slope parts of tributaries as it has been derived from wild resident spawners located in the upper Dranse system, upstream to impassable dams. According to Northcote (1992) and Ferguson (2006), the populations of brown trout located upstream to impassable dams show a strong tendency for the residency. In favour of this hypothesis, several sites of the upper Dranse basin stocked with the Mediterranean broodstock used in present study, (Caudron et al. 2006; Caudron 2008) showed the apparition of Mediterranean alleles 170, 192 and 194 in the wild resident population and, therefore, a contribution of stocked Mediterranean fish just a few years after the stocking. So, the offspring of the sedentary Mediterranean broodstock could have had a poor aptitude to residency in the downstream, low-sloped part of these lake tributaries where they have to compete with high densities (50–100, 0+/100 m<sup>2</sup>) of juveniles issued from wild migrants, which, in addition, could benefit of the advantage of prior residence.

Several other hypotheses can be proposed to explain the poor contribution of Mediterranean stocked fry to autumn/early winter runs of lake-migratory trout spawners observed here. First, the juvenile stocked could have left the lake and migrated downstream in the Rhône River. Second, they could have stayed in the lake or could have strayed among tributaries, due to poor homing ability. Third, the effects of domestication in the hatchery could also have induced a poor survival of stocked individuals in Lake Geneva and/or a loss of the migration and reproductive behaviour. Negative effects of domestication on stocking efficiency and on integrity of wild populations are indeed numerous as indicated in a recent review (Kostov 2009). To complete the present results, lake-migratory trout should also be sampled directly in the lake to know if the stocked Mediterranean alevins produce lake-migratory trout caught by fishing before the stage of the spawning migration. Indeed, in some Irish lakes, O'Grady (1984) found a dominant contribution of stocked trout to fish caught in the lakes by angling, and, in contrast, a dominant contribution of wild trout to the migratory spawners ascending the tributaries.

Clearly, additional studies are still needed to fully understand the genetic impact of the stockings with Mediterranean trout on both migratory and resident life-history forms of brown trout of the Lake Geneva system.

Few studies report the monitoring of stocking experiments using a native Mediterranean population of brown trout. In the Dranse River system (see above), Caudron (2008) and Caudron et al. (2006) found an increase of the Mediterranean alleles in the admixed populations following the stocking of native Mediterranean trout. In Slovenia, nearly pure populations of the threatened *Salmo marmoratus* were used to install new populations in isolated sections, which were devoid of fish (Crivelli et al. 2000). The results of these experimental introductions showed that such a stocking programme could successfully establish new populations in isolated upstream-sections of rivers (Vincenzi et al. 2008). Furthermore, Hansen et al. (2006) reported successful restoration of an admixed brown trout population by a supportive breeding program using remaining nonadmixed individuals.

In comparison, several previous genetic studies analysed the effects of stocking using nonnative domesticated hatchery strains of Atlantic origin on wild resident or migratory Atlantic brown trout populations. Some studies showed considerable gene flow between hatchery and wild migratory brown trout populations (Skaala et al. 1996; Palm et al. 2003; Nilsson et al. 2008; Sønstebo et al. 2008a,b) while some others found little genetic impact of stocking practices to migratory populations (Hansen et al. 2000; Heggenes et al. 2002, 2006; Ruzzante et al.

2004). In the case of Atlantic hatchery trout being stocked into native Mediterranean populations, a great variability was observed in the extent of introgression in different populations, ranging from hardly detectable introgression of exotic genes following several decades of intensive introductions (Largiadèr & Scholl 1996) to nearly complete displacement of the native gene pools (Largiadèr & Scholl 1995).

Taking into account the studies mentioned above, the situation studied here is rather unusual (i) because of the presence of two life-history forms and two origins of brown trout in the area studied and (ii) because a Mediterranean strain, which is native to the upper part of the river systems, is used for stocking the downstream sections of the rivers inhabited by Atlantic trout of unknown (native or introduced) origin. Utter (2000) reviewed the patterns of sub-specific introgression in two salmonid genera of North America (*Oncorhynchus* spp.) and Europe (*Salmo* spp.). He found that freshwater resident populations were more susceptible than anadromous ones to introgression from genetically distinct lineages including some lineages that had been isolated for more than a million years. Thus, our findings are well in line with the general findings of this review.

It cannot be inferred from our results showing no contribution of stocked Mediterranean to lake-migratory trout populations that the latter represent native Atlantic populations. However, it suggests that Atlantic migratory trout represent well-established populations maintained by natural recruitment, which do not need to be supported by supplemental introductions. To elucidate the real contribution of the stocking, all the stocked trout in Lake Geneva and in the tributaries were recently marked and also new genetic studies are programmed to try to clarify the origin, natural or anthropic, of Atlantic genes in the lake-migratory trout of Lake Geneva.

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