Genetic risks of supplementing trout populations with native stocks: a simulation case study from current Pyrenean populations

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6   study from current Pyrenean populations

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25   **Abstract**

26   The risks of supplementation must be examined to assess the genetic effects to native

27   wild populations before full implementation or exclusion of programs that involve

28   captive breeding and release. Real genetic data can be applied to simulations of genetic

29   changes in populations of interest, and subsequently used in risk assessment. Ancestral

30   Mediterranean brown trout lineages exhibit complex population structure among native

31   populations. Genetically divergent Atlantic stocks were maintained and released in the

32   Mediterranean rivers as recreational fish, which resulted in hybridization and

33   introgression with local populations. Therefore we designed a new supplementation

34   program based on native stocks, and evaluated the genetic risks associated with

35   releasing native fish in recreational fisheries. Our simulation was delimited by the

36   observed population genetic structure, and available hatchery facilities in the study

37   region. Supplementation with native stocks maintained estimates of gene diversity

38   indexes (H_T, H_S and G_{ST}). However, simulations indicated that long-term

39   supplementation significantly reduced genetic diversity among locations due to a

40   homogenizing effect of populations along each management unit. Therefore, such

41   reinforcements compromised the conservation of local genetic variation. Nevertheless,

42   replacement of current foreign with native stocks can be an important step towards

43   promoting the value of preserving local diversity among anglers.

44   **Introduction**

45   Freshwater fish are among the most endangered species groups due to various

46   anthropogenic impacts, including habitat fragmentation and contamination, and water

47   exploitation and diversion (Freyhof and Brooks 2011). In addition, several freshwater

48   fish species have exhibited notable declines in recent decades from exotic species

49   introductions, overfishing, and/or release of non-native stocks (Cowx and Gerdeaux

50   2004). In temperate and cold river basins, salmonids have experienced declines due to

51   each of these threats on native freshwater biodiversity (Lewin et al. 2006; Naish et al.

52   2007), and on-going climate change has increased the vulnerability and endangered

53   status of salmonid species worldwide (e.g. Hari et al. 2006; Almodovar et al. 2012; Vera

54   et al. 2013). In addition to habitat recovery, several other strategies have been suggested

55   to improve local degraded populations. Extensive release of hatchery cultured fish to

56   enhance recreational opportunities has been widely applied, and supported by anglers

57   (Brown and Day 2002; Arlinghaus and Mehner 2005; Cowx et al. 2010). Despite the

58   widespread biological concerns of applying supplementation practices from hatchery-

59   reared fish to restore wild populations (Laikre and Ryman 1996; Lewin et al. 2006;

60   Naish et al. 2007; Araki and Schmid 2010), these practices continue; and anglers show a

61   positive response by increasing their fishing efforts soon after fish are released (e.g.

62   Baer et al. 2007), which subsequently promotes revenues to local economies

63   (Arlinghaus et al. 2002).

64

65   In European basins, brown trout are an integral target for a socio-economically

66   important recreational fishery (Elliot 1989). The economic value of the fishery

67   mandates the development of management strategies focused on achieving and

68   maintaining the delicate balance between exploitation and conservation of the resource

69   (Araguas et al. 2009; Arlinghaus et al. 2010; Cowx and Van Anrooy 2010). Due to a

70   reduction in the abundance of catchable trout, anglers perceived efforts to reinforce

71   populations as a positive management objective (Arlinghaus and Mehner 2005). Such

72   circumstances also promoted supplementation initiatives among fisheries managers,

73   because recreational fisheries were traditionally managed based on the quality of the

74   fishing experience (Cowx and Gerdeaux 2004), and trout anglers associated quality with

75   catch quantity, and satisfaction followed (Arlinghaus and Mehner 2005). However,

76   management decisions based on anglers' perception could perpetuate stocking as a

77   panacea to maintain both, sustained fishery and the anglers' satisfaction, but at the risk

78   of replacing local fish diversity by hatchery stocks (van Poorten et al. 2011).

79   Mediterranean brown trout populations have been extensively supplemented with

80   North-eastern Atlantic hatchery stocks (hereafter, foreign stocks), resulting in

81   introgressed populations, and homogenization among wild populations (Poteaux et al.

82   1999; Marzano et al. 2003; Jug et al. 2005; Sanz et al. 2006; Apostolidis et al. 2008).

83   For example, Araguas et al. (2004) reported extensive foreign stock releases

84   compromised the genetic integrity and differentiation among native trout populations in

85   eastern Pyrenean rivers. In this region, up to 8 million fish were released in year 1995,

86   but later hatchery releases lowered and have been stabilized in recent times to 2 million

87   fish per year (Araguas et al. 2008). Because local salmonid populations often exhibit a

88   fitness advantage relative to foreign populations (Fraser et al. 2011; Perrier et al. 2013),

89   supplementation with foreign stocks is likely to reduce the mean population fitness in

90   recipient locations due to outbreeding depression and maladaptation of released fish

91   (Rhymer and Simberloff 1996, Baskett et al. 2013). Studies on the adaptive value of

92   local brown trout differences remain scarce, and not all phenotypic traits respond

93   similarly. For example, Jensen et al. (2008a) reported local adaptive variation for length

94   at hatching, and length at first feeding among four Danish trout populations within close

95   geographic proximity. However, adaptive evidence for distinct hatching and survival

96   times was not detected among five brown trout populations in the Swiss Rhine Basin

97   (Stelkens et al. 2012). Nevertheless, a precautionary approach in conservation and

98   fisheries management mandates the preservation of local types, at least until clear

99   scientific evidence is collected to assess the biological significance of local differences

100 (e.g. FAO 1995).

101

102 European brown trout have several evolutionary lineages (revised in Kottelat and

103 Freyhoff 2007). The Atlantic one (now considered as *Salmo trutta* sensu stricto in IUCN

104 red list) was native in the Atlantic river basins northward from the Pyrenees. Several

105 morphological and genetically distinct Mediterranean lineages have been described (e.g.

106 *S.rhodanensis*, *S.cettii*, *S.macrostigma*), and some of them are now identified as

107 threatened species in the IUCN red list (Freyhoff and Brooks 2011). However, a

108 taxonomic revision of the trout types inhabiting Mediterranean Iberian rivers is still

109 lacking; despite they could represent several unnamed species (Kottelat and Freyhoff

110 2007). As a result, the Spanish inland fisheries agencies consider all trout populations in

111 Spain as *S.trutta*, according with former taxonomic studies (Lelek 1980). However,

112 since the 1980s, the Autonomous Government of Catalonia, as well as other regional

113 administrations, recognized declines in endemic trout biodiversity for the region; and

114 since 1987 introduced management measures to recover native populations. These

115 measures included an increase in length requirement for catchable fish from 18 to 22

116 cm, a reduction in hatchery releases, which are now banned in some upstream stretches

117 declared as genetic refuge locations to protect native gene pools, and a change to catch

118 and release management in some previously fished areas (Araguas et al. 2009).

119   However, such restrictive measures typically received low angler support (Arlinghaus

120   and Mehner 2005). In addition, on-going assessments indicated that establishment of

121   genetic refuge did not result in a significant decrease in foreign stock alleles in the

122   Mediterranean Spanish and French wild populations (Araguas et al. 2008, 2009;

123   Caudron et al. 2011, 2012). Therefore, complementary active measures, including non-

124   native fish removal, supplementation with local native breeding stocks, and/or

125   translocation of wild native individuals were required to mitigate the genetic effects of

126   past releases with foreign fish (Caudron et al. 2012; Vera et al. 2013).

127

128   Hatchery strains derived from regional or local sources (hereafter, native stocks) have

129   been used to reinforce wild fish populations when conservation and fisheries

130   management goals were in common, because these stocks preclude damage to local

131   populations. For example, increased production with limited ecological and genetic

132   effects was observed in Hamma Hamma River steelhead (*Oncorhynchus mykiss*)

133   populations following supplemental stocking (Berejikian et al. 2008; Van Doornik et al.

134   2010). Hess et al. (2012) detected minimal negative impacts on fitness in a Chinook

135   salmon (*O. tshawytscha*) population in the Columbia River enhanced with supportive

136   captive breeding. Native stock reinforcement following habitat recovery is an accepted

137   measure within the European Inland Fisheries Advisory Commission (EIFAC) code of

138   practice for recreational fisheries (FAO 2008). Nevertheless, released fish from native

139   stocks and their wild progeny often exhibited decreased performance compared to wild

140   populations (reviewed in Araki et al 2008). For instance, Christie et al (2012) reported

141   genetic changes in the steelhead (*O.mykiss*) induced during a single generation of

142   hatchery culture resulting in maladaptation to the wild. Consequently more empirical

143   data is needed to evaluate the genetic and fitness effects of supplementation from native

144   stocks (Naish et al 2007; Fraser 2008; Araki and Schmid 2010).

145

146   The risks inherent with a supplementation program involving captive stocks must be

147   carefully assessed prior to implementation of the program with native populations

148   (Naish et al. 2007; FAO 2008). Theoretical studies demonstrated reduced Ne and fitness

149   of wild populations following supportive breeding (Wang and Ryman 2001; Ford

150   2002), and a decline in local populations following long-term supplementation by native

151   domestic stocks (Satake and Araki 2012). The effect occurred through density-

152   dependent overcompensation during recruitment, resulting in fewer wild fish recruits.

153   Simulations from real genetic data, augment these theoretical population genetics

154   approaches (Hoban et al. 2012), which facilitated assessments of genetic changes in

155   populations of interest (e.g. Perrier et al 2013).

156

157   In the present study, we assess patterns of genetic diversity within and among

158   populations at 13 sampling locations along four river basins in the Pyrenean Mountains

159   as a basic pre-requisite to design a regional native hatchery stocks program. We

160   subsequently simulated a supplementation program that involved replacement of foreign

161   by native trout stocks using the observed population structure to select source locations,

162   while integrating the existing regional hatchery facilities and hatchery personal

163   expertise. We assessed the long-term genetic effects of the releases, including the target

164   population gene pools but also the population structure at the intra- and inter-basin

165   levels. Finally, we discussed the social and economic benefits of the results relative to

166   regional trout fisheries.

167

168   **Materials and methods**

169   *River network, sampled locations and estimates of genetic diversity*

170   In the Spanish eastern Pyrenees, the river network is organized into two major units for

171   water supply and hydroelectric production, which includes the Ebro River basin and the

172   coastal rivers. The Ebro basin includes the easternmost Segre River drainage, composed

173   of the mainstem, and its tributaries the Noguera Pallaresa River (2820.1 km^2 drainage

174   surface), and the Noguera Ribagorzana River (2045.6 km^2 drainage surface). The Segre

175   basin supports the most preserved native populations of Mediterranean brown trout in

176   the Iberian Peninsula (Sanz et al. 2002). The largest coastal rivers are the Llobregat

177   (4948.3 km^2) and Ter (3010.5 km^2) Rivers. Trout populations only inhabit the

178   headstreams of these rivers, with trout habitats more abundant in the Ter River, with

179   headstreams up to 2000 m a.s.l., where the Llobregat are below 1300 m a.s.l.

180

181   A total of 854 wild brown trout were collected during summer 2006 by electro-fishing

182   at 13 localities along the Noguera Pallaresa, Noguera Ribagorzana, Llobregat, and Ter

183   River basins (Table 1, Fig. 1) to assess patterns of genetic diversity within and among

184   Pyrenean populations. Each fish was anesthetized with tricaine methane-sulphonate

185   (MS-222) to biopsy a piece of the adipose fin. The sample was stored in an eppendorf

186   tube containing 96% ethanol and transported to the laboratory until DNA extraction. In

187   the field, the fork length of each fish was measured (to the nearest 0.5 cm) to estimate

188   fish age (0+, 1+, 2+, 3+, and older fish) based on FISAT II software (Gayanilo et al.

189   2005) modal progression analysis and length-age relationships provided by Rocaspana

190   et al. (2006) for Pyrenean trout populations. Once recovered from anaesthesia, fish were

191   returned to the streams alive. We also analysed 96 fish from the 2003-year cohort of the

192   foreign stock at Bagà hatchery (HAT1, see Fig1) used to reinforce Pyrenean brown

193   trout populations in the region. We only analysed fish from this hatchery because trout

194   culture at HAT2 is discontinued when trout production at HAT1 is enough to

195   supplement regional fisheries. Only when an increased hatchery production is required,

196   HAT2 receives fish from HAT1 to regenerate a new stock.

197

198   Genomic DNA was extracted from fin tissues using a proteinase K-Chelex extraction

199   protocol to assess genotypes for nine polymorphic microsatellite loci (*Str15*, *Str73*,

200   *Str591INRA*, *Ssa85*, *Ssa408*, *SsHaeIII14.20*, *SsoSL417*, *SsoSL438*, and *SSsp2213*) as

201   described by Sanz et al. (2009). MICRO-CHECKER 2.2.1 (Van Oosterhout et al. 2004)

202   revised microsatellite genotypes to test large allele dropout and null alleles.

203   Conformance of genotype distributions with Hardy-Weinberg expectations were tested

204   by exact probability tests (Guo and Thompson 1992) using the computer package

205   GENEPOP 3.3 (Raymond and Rousset 1995). For each location, the minimum number

206   of homogenous units ($K = 1, 2$, and 3) was determined using the Bayesian Markov

207   Chain Monte Carlo (MCMC) approach in STRUCTURE 2.3.3 (Pritchard et al. 2000) as

208   indicated in Sanz et al. (2009). FSTAT2.9.3 software (Goudet 1995) was used to

209   summarize genetic diversity within samples as follows: mean unbiased expected

210   heterozygosity (H_E), mean direct count heterozygosity (H_O), and average allele richness

211   per locus (A_R). To measure the level of current foreign stock introgression in each wild

212   location, we estimated the average proportion of foreign stock ancestry (q) following

213   Sanz et al. (2009), however Araguas et al. (2008) already reported estimates for 10 of

214   these locations based on *LDH-C** locus polymorphisms. Effective population size (Ne)

215   at each study location was estimated using linkage disequilibrium between loci in the

216   LDNe 1.31 program (Waples and Do 2008). *Ne* was estimated by removing rare alleles

217   with frequency less than 0.02, due to a sample size of less than 50 fish in some study

218   locations. The LDNe method assumes discrete generations, which are not the case in

219   brown trout; but the method roughly estimates Ne when the number of sampled cohorts

220   approximates the suspected generation time (Waples and Do 2010). At our study

221   locations, three cohorts (0+, 1+, and 2+, see results) were the most abundant, as

222   similarly reported in the Iberian Peninsula (Lobón-Cervia et al. 1986; Nicola et al. 2008;

223   Parra et al. 2009), suggesting generation lengths between 2-3 y for these trout

224   populations. Additional confidence on estimated Ne from our locations resulted from

225   genetic stability reported among five consecutive cohorts (year 1998 to 2002) studied in

226   an earlier work in the Vallfarrera stream (Vera et al. 2010).

227

228   *Population structure*

229   Genetic divergence between locations was examined using a matrix of pairwise genetic

230   differentiation, F_{ST} (Weir and Cockerham 1984), and its significance (based on 1000

231   permutations) in FSTAT. Patterns of genetic diversity within and among the study river

232   basins were quantified by gene diversity analyses (Nei 1987) using FSTAT and

233   Analysis of Molecular Variance (AMOVA) using Arlequin 3.5.1.3 (Excoffier and

234   Lischer 2010). Hierarchical levels followed hydrogeographical criteria within (F_{SC}) and

235   among (F_{CT}) basins or tributaries, and involved an analysis spanning the entire study

236   region, and separate analyses for each river basin. Additionally, the pairwise genetic

237   distance matrix among samples (D_a, Nei 1987) computed using MSA 4.05 (Dieringer

238   and Schlötterer 2003) was used to generate a multidimensional scaling (*MDS*) using

239   SPSS program to depict genetic similarity between study locations. Finally, we

240   examined the most likely number of genetically homogeneous groups ($K = 1$ to 13) in

241   the study region from the Bayesian method implemented in STRUCTURE 2.3.3

242   following Evanno et al. (2005) after 20 replicate STRUCTURE runs for each K value.

243   Each run used a burn-in of 10000 iterations, a run length of 10000 iterations, and all

244   other parameters set to default model.

245

246   *Design of the native stocks*

247   The current foreign hatchery stock originated from central European trout, and regional

248   fishery management could require its replacement with native stocks in the future.

249   However, we should keep in mind that despite the stock origin, the current normative

250   framework involving hatchery trout stocks in the study region serves to support

251   recreational fisheries, and not conservation genetics goals. In addition, legislation on

252   other water uses (e.g. industrial, agricultural, and domestic consumption, or

253   hydroelectric production) often takes prevalence over recreational fisheries. The

254   Environmental Services of the Autonomous Government of Catalonian manage trout

255   populations in all the regional basins. However, water supplies and hydroelectric

256   production in the Ebro River basin are under the jurisdiction of the C.H.E.

257   (Confederación Hidrográfica del Ebro), a Spanish State agency; while in the coastal

258   rivers are jurisdiction of the A.C.A (Agència Catalana de l'Aigua), an agency of the

259   Autonomous Government of Catalonian. Because our genetic results suggest "basin" as

260   a primary source for population structure, we focused on "basin" (Ribagorzana,

261   Pallaresa, Llobregat and Ter River basins) as a work unit, which also facilitates the

262   administrative collaboration between the environmental and water agencies. The

263   location (see Fig.1) and facilities of the two regional hatcheries should easily

264   accommodate and separately manage several stocks, despite their previous expertise,

265   which focused on one stock.

266

267   For the simulation, we first selected a source location for each river basin, which was a

268   challenging task (Allendorf and Ryman 1987; Griffith et al. 2009; Laikre et al. 2010).

269   The selection criteria involved Hardy-Weinberg and gametic equilibria; and low

270   impacts involved with on-going hatchery release of the foreign stock (see results in

271   Table 1). Furthermore, genetic refuge locations were preferred (Araguas et al. 2008).

272   We finally prioritized sources located on river drainage mainstems, because brown trout

273   in mainstem populations were typically larger than in small tributaries (Parra et al.

274   2009), which can increase offspring abundance in the first generation at hatchery

275   facilities, i.e. large trout should have increased fecundity (Lobón-Cerviá et al. 1997).

276   Abundant progeny from as many parents as possible might facilitate further F1

277   manipulation to prevent undesirable genetic diversity losses in hatchery stocks (Araki et

278   al. 2008, Fraser 2008). A large amount of stock diversity was also desirable to prevent

279   further changes by selection and domestication (Fraser 2008; Araki and Schmid 2010).

280   Because of the high amount of foreign stock introgression in the single location studied

281   at the Llobregat River basin we decided to join this basin to the Ter River for the

282   simulated supplementation program as a single management unit for all coastal basins.

283   Subsequently, three captive native stocks were simulated, one to stock the Noguera

284   Ribagorzana River, one the Noguera Pallaresa, and one to stock the coastal basins.

285   According with results on genetic structure, the simulated native stocks supplementation

286   program allowed assessment of genetic effects at intra and inter basins scales covering

287   aspects as: (i) maintenance of the population structure at regional scale, (ii) preservation

288   of local singularities, and (iii) recovery of native gene pools in heavily introgressed

289   locations with foreign alleles. In this later case, at Filià location we simulated the

290   recovery using a native stock derived from a location in the same river, but at the

291   Riutort location by using an stock originated from distinct river.

292

293   *Simulated supplementation program*

294   Our simulation evaluated the long-term effects (100 generations) on genetic population

295   structure of a potential supplementation program using the three native stocks

296   characterized above. Initially, a *Ne* of 100 was assumed for each location, well in the

297   range of estimated brown trout *Ne* at each study location (see results in Table 1). The

298   supplementation effort was adjusted to accept the one-migrant-per-generation (OMPG)

299   rule, considered enough to maintain polymorphism and heterozygosity levels within

300   wild locations, while facilitating divergence (Mills and Allendorf 1996). This level of

301   hatchery introgression per generation was consistent with previous estimates in the

302   study area resulting from release of foreign hatchery fish (1 to 5%, Araguas et al. 2004).

303   For each study location using HYBRIDLAB software (Nielsen et al. 2006), we first

304   simulated samples of 100 individuals at generation 0 from current allele frequencies. In

305   addition, for each native stock we simulated 1000 individuals from the current

306   genotypes representing the source locations. Although a *Ne* size of 1000 fish for stock

307   might be perceived as unrealistic due to the abundant evidence for genetic changes in

308   salmonid hatchery stocks (Naish et al. 2007; Araki and Schmid 2010), it allowed us to

309   address genetic change in wild populations resulting from fish released from native

310   stocks kept genetically similar to the native source. In addition, this approach might also

311   have utility in evaluating genetic change induced by repetitive supportive breeding from

312   a few local sources.

313

314   All wild and stocked fish simulated at generation 0 were aged as adults, and randomly

315   sexed as male or female. Input data were subsequently converted in an FSTAT format

316   file used for simulations in NEMO v2.2 software (Guillaume and Rougemont 2006). In

317   each management unit (Noguera Pallaresa, Noguera Ribagorzana and Coastal basins),

318   and in each of their wild population, we simulated 100 generations of native stock

319   reinforcement by setting the local immigration rate (m) from the assigned stock to 0.01

320   using the NEMO software "breed-disperse" life cycle event. Ne for wild and stock

321   populations was maintained each generation at 100 and 1000 individuals, respectively.

322   All loci assorted independently (recombination rate adjusted to 0.5), and simulated

323   genotypes at each generation were stored for further analyses.

324

325   The above reinforcement scenario was compared with simulated management in the

326   absence of supplementation, and drift as a single evolutionary force during 100

327   generations based on negligible dispersal and gene flow reported between resident trout

328   populations even at hydrogeographic distances of a few kilometres (Knouft and Spotila

329   2002; Vera et al. 2010; Vollestad et al. 2012). Gene pool evolution within each location

330   was subsequently simulated as indicated above for the supplementation program, but

331   local immigration from the assigned stock was not permitted (m = 0). Additional

332   simulations were performed for Ne of 50, and 1000 fish in wild populations for both

333   scenarios, with and without supplementation with native stocks. Finally, three

334   additional data sets were simulated based on maintaining current foreign stock releases

335   into all wild locations (m = 0.01 per generation), and respectively considering Ne of 50,

336   100, and 1000 fish. In summary, 3 x 3 (three scenarios x three Ne) simulated genotype

337   data sets were collected.

338

339   Genetic diversity levels at Generation 0 and 100 for each location and scenario were

340   estimated using FSTAT. In all simulations, gene pool stability between generations 0

341   and 100 in each wild location, and in each simulated stock, was estimated by F_{ST} values

342   (Weir and Cockerham 1984). Significance levels were obtained from 1000 permutation

343   tests in FSTAT. Patterns of population structure among locations followed after 100

344   simulated generations, and for each scenario were estimated by gene diversity analyses

345   (FSTAT). The proportion of divergence between locations within (SC) and among (CT)

346   river basins was examined using hierarchical AMOVAs (Arlequin Software). In

347   addition, population relationships among locations in simulated scenarios (with and

348   without reinforcement) were depicted by nonparametric multidimensional scaling

349   (MDS) from the *Da* genetic distance matrix between location pairs, as indicated above

350   in the study of captured wild samples. Furthermore, to assess local genetic changes in

351   scenarios with and without supplementation with native stocks, and *Ne* of 100, we

352   estimated the remaining proportion of the simulated generation 0 and the contribution of

353   the native stock , both as *q*-values following Sanz et al. (2009) as indicated above for

354   estimating the current contribution of the foreign hatchery stock on wild locations. We

355   should note that the estimated native stock *q*-value associated with each location under

356   drift was not related to any effects of fish release, but to historical evolutionary

357   processes within and among basins, driving the current genetic divergence between

358   locations. The estimated native stock *q-value* under drift provides only a reference value

359   to compare with *q*-value obtained under reinforcement.

360

361   **Results**

362   *Genetic diversity and population structure of wild populations*

363   Brown trout populations at the study locations were short-lived, with a clear dominance

364   of age groups 0+ to 2+, with the exception of the Vallter location, where older fish (>

365   2+) were also abundant (Table 1). On average, 77.0 % of fish sampled were in age

366   classes 0+ to 2+, and ranged from 55.4% in Vallter to 99.5% in Nuria. Genetic diversity

367   differed among locations (Table 1), with the highest variability exhibited in Filià

368   (A_R=8.40, H_E=0.769), and the lowest variability in Vallter (A_R=3.23, H_E =0.393).

369   Genetic variability in the current hatchery stock was also high (A_R=7.78, H_E =0.682),

370   and some abundant allele variants detected in this foreign stock (*Str73*146*,

371   *Str591INRA*158*, *SsHaeIII14.20*312*, *SsHaeIII14.20*324*, *SsHaeIII14.20*322*, and

372   *SsoSL438*105*) were rare in wild trout.

373

374   Following Bonferroni correction, genotype distributions at three study locations (Tor,

375   Erta, and Nuria) deviated from Hardy-Weinberg expectations due to homozygote excess

376   (supplementary Table S1). MICRO-CHECKER suggested the presence of null alleles in

377   the Nuria *Str15INRA* locus, and the Tor *SsoSL417* locus. Nevertheless, evidence of null

378   alleles at these two loci was not detected at other study locations. In Nuria, Riutort, Tor,

379   Filià, Cavallers, and Erta, significant gametic disequilibria was observed after applying

380   a Bonferroni correction for multiple tests. High hatchery ancestry (*q*-values) were

381   detected in fish sampled at Riutort and Filià (0.30 at both study locations) (Table 1), but

382   in the other study locations the estimated hatchery ancestry for captured fish was below

383   5% (0.05). STRUCTURE results suggested two genetically distinct units presented in

384   Riutort, Filià, Nuria and Tor locations. The abundance of rare homozygotes for alleles

385   common among hatchery fish indicated recent releases in these streams responsible for

386   observed Hardy-Weinberg and gametic disequilibria. Estimated *Ne* ranged from 18.7 in

387   Filià to 293.0 in Ermita, and a larger indeterminate estimate in Ainet, i.e. 243.9 to α.

388   Reduced *Ne* were related to locations with evidence for recent release of hatchery fish

389   (*K* = 2; Table 1).

390

391   Significant pairwise differentiation was detected in all but one comparison, *i.e.* Ermita-

392   Palomera (Table 2). Despite high hatchery *q*-values observed in Riutort and Filià, all

393   wild populations exhibited significant genetic differentiation from current hatchery

394   foreign stock (F_{ST} range 0.116-0.426). Hierarchical AMOVA results for the entire study

395   area reflected higher significant divergences within (*SC* component = 86.14%, Table 3)

396   than among basins (*CT* component = 13.86%). Due to the increased number of study

397   locations in the Pallaresa and Ribagorzana basins, a hierarchical AMOVA was possible

398   for each of the two basins. In both basins, results showed increased differentiation

399   within compared to among tributaries. In the Pallaresa River (G_{ST} = 0.160), where the

400   two more hydrogeographically separated study locations were Palomera and Filià (91.6

401   km), only 23.35 per cent of the total differentiation was assigned to differences among

402   tributaries. In the Ribagorzana River, increased differentiation was observed among

403   locations (G_{ST} = 0.357), even though within this basin, 64.95% of the population

404   differentiation was assigned within tributaries. In this basin, the largest hydrogeographic

405   separation among study locations occurred between Conangles and Cavallers, 50.1 km

406   apart.

407

408   STRUCTURE (Fig. 2) and MDS analyses based on D_a distances (Fig. 3, 2006

409   collections) were largely congruent. Gene pools from study locations were grouped

410   following a hydrogeographic pattern (Ter, Pallaresa, and Ribagorzana), however two

411   outlier samples from different basins were grouped together, *i.e.* Manyanet and Erta,

412   and a fifth group comprised the high hatchery ancestry locations Riutort and Filià (*q*-

413   value = 0.300, see Table 1).

414

415   *Simulated long-term effects of supplementation with native stocks*

416   None of the study locations had the complete requirements to be an ideal source

417   location for native stocks (Table 1). The selected source locations for simulated native

418   stocks were then determined as follows: Ermita for the Noguera Pallaresa basin due to

419   the location inclusion as a genetic refuge, reduced average hatchery ancestry, and lack

420   of evidence for Hardy-Weinberg or gametic disequilibria; Conangles for the Noguera

421   Ribagorzana River due to its location in a genetic refuge area in the river mainstem, and

422   Vallter for Coastal basins due to low incidence of hatchery releases, the largest Ne

423   estimate among the three study locations in the coastal management unit, and inclusion

424   as a genetic refuge. Permutation tests in FSTAT indicated that average genetic diversity

425   from source locations (see Table 3) was not significantly different from the average

426   diversity at other study locations ($P > 0.05$), and divergence among source locations

427   (G_{ST}) resulted in the observed population differentiation among the eastern Pyrenean

428   trout populations ($P > 0.05$). Despite Ne for stocks fixed at 1000 fish, small but

429   significant ($P < 0.05$) F_{ST} value between generation 0 and 100 was observed in all

430   simulated stocks (including the foreign one). However, these allelic changes did not

431   significantly modify stock diversity levels (allele richness and gene diversity) as

432   indicated by FSTAT permutation tests.

433

434   Following 100 generations, simulated genetic drift in wild populations of 100

435   individuals caused loss in genetic diversity, and significant allele frequency changes (P

436   < 0.001) at all locations, indicated by F_{ST} coefficients (Table 4). Simulations showed

437   supplementation with native stocks maintained levels of diversity (heterozygosity and

438   allele richness) at most locations, but again, significant F_{ST} values were detected ($P <$

439   0.001), even at the native stock source location. However, genetic changes resulting

440   from supplementation with native stocks were generally less severe (decreased F_{ST}

441   value) than alterations observed from maintaining supplementation with the current

442   exogenous stock (Table 4). Short time supplementation with native stocks (10

443   generations) did not significantly alter diversity patterns within and among locations

444   (Table 3, Fig. 3 G10 N100). Estimates of ancestries in the populations following 100

445   generations in scenarios with and without (drift) supplementation with native stocks

446   added information on genetic changes occurred in each location (Table 5). Despite

447   losses in genetic diversity for simulations involving genetic drift, the estimate remaining

448   ancestry for the local original (generation 0) gene pool was close to 1.0 at each location,

449   and the estimated native stock ancestry was only high for the respective source location

450   except in Palomera. Here, the estimated native stock ancestry was high because of its

451   current genetic similarity with the Ermita trout, which was used as source for the

452   simulated native stocks in the Noguera Pallaresa River basin ($F_{ST} = 0.003$; Table 2).

453   However in all basins, supplementation with native stock produced declines of the

454   original gene pool ancestry for all locations, particularly in locations currently showing

455   large genetic divergence from the source population (Tor and Manyanet in the Noguera

456   Pallaresa basin; Cavallers, Nicolau, and Erta in the Ribagorzana basin, and Riutort in

457   the coastal basins). As expected, these declines were related to increased estimates in

458   native stock ancestry. Altogether indicated that supplementation with native stocks was

459   efficiently eroding local gene pools.

460

461   In simulations of *Ne* of 100 individuals, FSTAT comparisons confirmed reduced

462   average allele richness within locations in the simulated scenario with and without

463   (drift) native stocks supplementation (Table 3). The simulation without supplementation

464   maintained total diversity (H_T) in the region by significantly reducing local diversity

465   (H_S), and increasing divergence (G_{ST}) among locations. The native stocks

466   supplementation program showed reduced effects on gene diversity indices (H_T, H_S and

467   G_{ST}), as significant changes were not detected with current estimates. In addition,

468   continued release of foreign stock did not result in significant changes in allele richness

469   and gene diversity indices. However, hierarchical AMOVAs indicated releases from

470   foreign stock into all native population locations nearly eliminated the genetic

471   distinction among management units (CT component close to 0; Table 3). Hierarchical

472   AMOVAs also showed that without supplementation, drift alone was not sufficient to

473   completely erode genetic differences within (SC component) and among (CT

474   component) into the management units. Nevertheless, supplementation with native

475   stocks resulted in increased genetic homogenization of populations within management

476   units (strong reduction in the SC component). MDS results from *Da* genetic distance

477   matrices between locations (Fig. 3) clearly depicted all changes in population structure

478   in the study area resulting from supplementation with exogenous and native stocks.

479   Consequently, following long-term supplementation by native stocks, populations were

480   clustered based on management units, while maintaining releases from a common

481   foreign stock, resulted in genetic relationships among populations from distinct

482   management units.

483

484   In simulations involving population sizes of 50 fish, the effects of drift were evidenced

485   by reduced average allele richness and increased divergence among locations under all

486   simulation scenarios (Table 3), however a large "among management units" component

487   (CT) was obtained from supplementing with native stocks (Fig. 3). This diversity

488   component (CT) was not preserved in the other two scenarios (with or without

489   supplementation with the foreign stock). Nevertheless, simulations with population

490   sizes of 1000 fish exhibited decreased genetic changes in gene diversity and hierarchical

491   population structure under the drift scenario compared to any supplementation program

492   (Table 3).

493

494   **Discussion**

495   *Current wild population status*

496   Brown trout in the eastern Pyrenean rivers showed levels of diversity at microsatellite

497   loci well within the range detected in Mediterranean populations from other countries

498   (Jug et al. 2005; Apostolidis et al. 2008). Populations showing the greatest genetic

499   impacts following releases of the Atlantic foreign stock (Riutort and Filià locations,

500   Table 1) also exhibited the highest estimates of heterozygosity. Recent foreign stock

501   releases remained common evidenced by the two breeding units detected at four study

502   locations (Nuria, Riutort, Tor, and Filià), which were supported by high estimates of

503   hatchery ancestry or Hardy-Weinberg and gametic disequilibria. Despite estimated

504   introgression rates below 5% from all study locations, with the exception of Riutort and

505   Filià, wider geographical surveys suggested average introgression rates from Atlantic

506   foreign stocks exceeded 10% in eastern Iberian trout populations (Sanz et al. 2002;

507   Aparicio et al. 2005; Almodovar et al. 2006). Recent releases likely contributed to the

508   small Ne estimated in Tor and Filià, as expected from theoretical predictions on

509   hatchery releases and long-term supportive breeding programs (Ryman and Laikre

510   1991; Wang and Ryman 2001, Waples and England 2011). However, a large amount of

511   native genetic diversity was still preserved within the eastern Pyrenean rivers, where

512   native brown trout remained morphologically distinct from hatchery fish (Aparicio et al.

513   2005).

514

515   *Ne* estimates suggested adequately self-sustained trout populations in some locations,

516   including Ter, Palomera, Ermita, Ainet, Manyanet, Nicolau, and Erta. In the Iberian

517   Peninsula, age at maturity for brown trout is between 1+ and 2+ for females, and 2+ to

518   3+ for males (Lobón-Cerviá et al. 1986; Parra et al. 2009). The adult fish older than 2+

519   at time of capture represented less than 40% in all study locations with the exception of

520   Ter. In addition, fish available for angling should be even less abundant, because at the

521   study locations, the oldest fish (> 3+) reach catchable length (22 cm) *i.e.* in Iberian

522   rivers (Lobón-Cerviá et al. 1986; Rocaspana et al. 2006; Parra et al. 2009). A decrease

523   in catchable fish (< 10%) abundance is common among fished trout populations

524   compared with increased abundance in unfished stretches (Almodovar and Nicola

525   2004). Nevertheless, environmental factors and anthropogenic-mediated perturbations,

526   in addition to angling contribute to reductions in catchable trout throughout Spanish

527   rivers (Almodovar and Nicola 1999; Nicola et al. 2009; Ayllón et al. 2012), including

528   Pyrenean waters (Garcia de Jalon et al. 1988, 1996; Alonso et al. 2011). Cumulatively,

529   these factors promote social support for supplementation practices.

530

531   *Genetic effects of the supplementation with native stock*

532   Our results indicated that following short-term supplementation, sporadic supportive

533   breeding programs from local sources would not markedly change the gene pools of the

534   supplemented populations. In addition, as Caudron et al. (2011, 2012) reported in

535   French brown trout populations following supportive breeding programs, simulations

536   predicted recovery of native diversity in the Filià and Riutort populations highly

537   impacted by current releases of a foreign hatchery stock. In the French streams, trout

538   densities increased 20- to 55-fold, and the majority of juvenile trout (78–89%) were

539   first-generation descendants of released trout. However, recovering native alleles in

540   these previously highly introgressed populations might not restore native local ancestry,

541   but result in genetic swamping of the local populations along the management unit by

542   the native stock gene pool. Swamping was particularly relevant in locations reinforced

543   with a native stock originated from a source in other river basin as Riutort in our

544   simulations; in this location a notorious reduction of the original local ancestry was

545   observed. Within management units, such an effect was even more evident in

546   genetically unique native populations, as Manyanet and Erta in our study. In these

547   outlier populations, the native local gene pool were reduced following long-term

548   supplementation, while a notable increase in the estimate native stock ancestry was

549   observed. The expected result following long-term supplementation with native stocks

550   was then a significant reduction in diversity among locations within management units.

551   However, in the study region, current genetic differentiation was related more with local

552   divergence within rather than isolation between basins. Similar results have already

553   been observed in the wild; for example, populations of Coho salmon (*Oncorhynchus*

554   *kisutch*) in the Puget Sound of Washington State that underwent extensive hatchery

555   propagation share more of their ancestry recently than they did historically (Eldridge et

556   al. 2009). Hansen et al. (2009) reported substantial local changes in Danish brown trout

557   populations following long-term supplementation with Danish stocks. Supportive

558   breeding efforts in French Mediterranean trout populations showed over short-term time

559   scales, the genetic and demographic effects were restricted to river stretches 2 km

560   downstream from the release locations (Caudron et al. 2012), however other evidence

561   indicated expansion of hatchery genes by hybridized fish occurring over longer periods

562   of time following releases (García-Marín et al. 1998; Allendorf et al. 2004; Araguas et

563   al. 2008; Perrier et al. 2013).

564

565   Fishery managers should be aware that simply maintaining global estimates of gene

566   diversity indices (H_T, H_S and G_{ST}) does not insure conservation of local genetic

567   variation. For example, significant changes were not detected in diversity indices for

568   supplementation simulations, either using current foreign or native stocks, despite losses

569   of native alleles in all locations. Therefore, native stocks do not mean local stocks, even

570   at short hydrogeographical distances. Increased genetic differentiation between

571   Pyrenean brown trout populations suggested isolated populations at hydrogeographical

572   separation of just a few kilometres. In fact, the only non-significant divergence detected

573   in the entire study was between Palomera and Ermita locations, 1.2 km apart along the

574   same mainstem of the Vallfarrera River. Hierarchical partitioning of genetic diversity

575   revealed another relevant source of divergence between tributaries in the same river

576   basin. For example, the Tor River sample was collected 6 km from the Ermita location

577   in a tributary of the Vallfarrera River, and the estimated F_{ST} value between Tor and

578   Ermita samples was 0.175.

579

580   *Selective forces*

581   Our study was based on microsatellite loci, which are typically considered selective

582   neutral markers. However, there might be adaptive and selective processes modulating

583   and modifying our observations. First, we should consider the adaptive value of trout

584   genetic singularities in the Pyrenean locations. Certainly it remains to be demonstrated,

585   yet the observed divergences at microsatellite loci among these trout populations were

586   congruent with distinctions based on protein coding loci variation reported earlier by

587   Araguas et al. (2004). Local adaptation in salmonids occurs at several spatial scales

588   (from a few to thousands of kilometers), with local populations often manifesting a

589   fitness advantage over foreign populations (Fraser et al. 2011; Perrier et al. 2013). In

590   brown trout, selective processes have been detected at small spatial scales for loci

591   related to immune systems, *i.e.* MHC or TAP (Hansen et al. 2007; Jensen et al. 2008b;

592   Keller et al. 2011), but also at microsatellite loci linked to functional loci and QTLs

593   (Meier et al. 2011). Therefore, the high divergence (F_{ST}) observed between wild

594   Pyrenean populations could be related with local adaptation. In this sense, the

595   divergence observed among locations within each management unit is often as high as

596   values reported among wild locations and the current foreign stock, questioning whether

597   the choice of a single native source for each management unit would be a successful

598   management decision. Unfortunately, a supplementation program involving all

599   requested and necessary native stocks to preserve all local genetically differentiated

600   populations at the intra- and inter- basin levels currently appears unfeasible due to the

601   reduced infrastructure (two hatcheries), and high economic costs of maintaining several

602   native stocks.

603

604   Selective domestication induced by culture conditions is often detected in hatchery

605   stocks (Araki and Schmid 2010). Putative diversifying selection between wild

606   populations and hatchery trout stocks (e.g. Hansen et al. 2010) can result in additional

607   risk due to reduced average fitness of reinforced wild populations, even at low fitness

608   differences between wild and hatchery fish (weak selection), facilitated for instance by

609   reiterated immigration of wild fish into captive stock (Ford 2002). Surprisingly,

610   releasing hatchery stocks phenotypically differentiated from wild populations might

611   result in less harmful effects because the phenotype divergence could be associated with

612   traits maladaptive to wild conditions, and hence strong selective pressures purging

613   released fish before the reproductive season (Baskett and Waples 2012; Baskett et al.

614   2013). In the study territory, the current hatchery stock is phenotypically divergent from

615   wild populations (Aparicio et al. 2005), and its foreign origin and long-time

616   maintenance in captive conditions might result in maladaptation to wild conditions.

617   Based on the simulation results of Baskett et al. (2013), such maladaptation could

618   justify the reduced estimates of hatchery introgression detected in Pyrenean trout

619   populations despite the long period of intensive releases (Sanz et al 2002, Aparicio et al

620   2005), and questions the suitability of replacing foreign stock by native. Nevertheless,

621   reduced impact of hatchery releases has been reported among North-European

622   populations phylogenetically close to source populations of our foreign stock (e.g.

623   Ruzzante et al. 2001).

624

625   *Conclusions: management prospectives*

626   Results of our simulations indicated that in wild populations comprised of an effective

627   population size of 100 individuals, genetic drift would result in significant changes in

628   the study basin gene pools during the next one hundred years. However, drift alone

629   might better conserve distinct populations among locations within management units.

630   Based on simulation predictions, an increase in effective population size to 1000

631   individuals decreased the likelihood of significant changes in the study basin gene

632   pools. In this case, within and among population changes were lower than those caused

633   by regional reinforcement policies. Such results suggested that habitat restoration to

634   insure large effective population sizes might be enough to protect native genetic

635   diversity. Sociological studies on German anglers indicated that limiting brown trout

636   management to improve or maintain good habitat quality would receive anglers'

637   support, primarily when catches were increased, and fishing experiences were positive

638   (Baer and Brinker 2010), but even then, a large proportion of anglers were not opposed

639   to supplementation practices (Arlinghaus and Mehner 2005). Therefore, avoiding

640   hatchery-releases might compromise angler support for further management measures.

641   In the eastern Pyrenean rivers, a combination of genetic refuges to protect native trout

642   diversity in some stretches, and stocking practices in other river sections undergoing

643   intensive fishing efforts, has favoured restrictive measures in a per day bag and

644   increased minimum catchable size limits, and additional fishing river stretches

645   designated as catch-and-release areas (Araguas et al. 2009). In addition, the current

646   normative framework involving trout populations in the Spanish eastern Pyrenean

647   serves to support recreational fisheries, and anglers' societies are the key stakeholders

648   and one of the main lobbyists in decision-making on regulatory measures in the

649   Pyrenean river basin. Because of pending taxonomic revision, any distinct trout taxa

650   from the study region were not included in the most recent red list of European

651   freshwater fish (Freyhoff and Brooks 2011), limiting conservationists' arguments

652   against extensive hatchery trout releases and translocations.

653

654   Often criticized due to the conservation risks on remnant biodiversity (Laikre et al.

655   2010), hatchery stock supplementation is maintained as a traditional fisheries practice,

656   because it is perceived as a prophylactic measure for human induced damage, including

657   fisheries itself, on wild populations (Arlinghaus et al. 2002). From a put-and-take fish

658   stocking point of view, which is maintained in some eastern Pyrenean river stretches, it

659   may be irrelevant which stocks, foreign or native, are used for release. In fact, current

660   foreign hatchery stocks are well-adapted to hatcheries, and hatchery personnel have

661   sufficient expertise to generate large output to enhance regional trout fisheries.

662   However, compared with maintaining supplementation from foreign stock,

663   supplementation from native stocks might serve as a much better balance between the

664   social benefits of angling and biological damage to native diversity in the regional river

665   basins. Together with measures that limit native releases to locations of intensive

666   fishing that preclude the survival of released fish before the spawning season, the

667   replacement of current foreign with native trout stocks provides an important

668　opportunity to promote the value of preserving local diversity among anglers, and with

669　the presently implemented genetic refuge policy, should be an additional step in the

670　albeit slow transition to a fisheries model focused on local self-sustaining trout

671　populations, and regional habitat management. Native stock development can stimulate

672　regional hatchery expertise and hatchery personnel to manage native fish, and facilitate

673　short-term supportive breeding programs to recover endangered native trout

674　populations.

675

676　**Acknowledgements**

683

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981

982   **Figure Captions**

983

984

985   Fig. 1. Geographic locations of the brown trout collections and the two hatcheries

986   (HAT) in the study region. Location codes are defined in Table 1.

987

988   **Fig. 2**. Current individual sample relationships indicated by STRUCTURE analysis

989   considering 2, 3, 4 and 5 genetic groups. Each individual is represented by a vertical bar

990   partitioned into segments according to the proportion of the genome assigned to each of

991   the identified clusters. Location codes are defined in Table 1.

992

993   **Fig. 3**. Multidimensional scaling (MDS) plots of population genetic structure from

994   captured collections (2006 collections), and from simulations without (drift), and with

995   supplementation by current foreign or native stocks. Circles represent Noguera Pallaresa

996   locations, triangles Noguera Ribagorzana, and Coastal management unit is represented

997   by squares. Location codes are defined in Table 1.

Table 1. Genetic diversity in study brown trout locations. N: sample size, *Adult*: percentage of fish older than 2+ at time of capture (class 0+ excluded), A_R: Allelic richness per population, H_O: mean direct count heterozygosity, H_E: mean unbiased expected heterozygosity, q: estimate average proportion of foreign hatchery stock ancestry, K: number of genetically homogenous units in the sample, Ne: estimated effective population size (CI: Jackknife on loci 95% Confidence Interval), *GR*: Genetic refuge area. Very large effective sizes are indicated when negative Ne was obtained from LDNe calculations (Waples and Do 2010). In bold, locations used as source of the native stocks (see text)

River	Location	stream	N	Adult (>2+)	A_R	H_O	H_E	q	K	Ne (CI)	GR
N. Pallaresa	Palomera (PL)	Vallfarrera	55	34.7	6.51	0.654	0.664	0.004	1	281.0 (99.6 – α)	Yes
	Ermita (ER)	Vallfarrera	46	36.4	6.73	0.683	0.663	0.003	1	293.7 (88.7 – α)	Yes
	Tor (TOR)	Tor	43	8.5	4.89	0.380	0.421	0.034	2	27.5 (10.4 -152.2)	No
	Ainet (AC)	Cardos	71	35.0	7.31	0.665	0.666	0.018	1	Very large (243.9 – α)	No
	Manyanet (MY)	Flamisell	104	8.5	4.86	0.577	0.594	0.007	1	227.1 (109.4 – 1877.4)	Yes
	Filià (FI)	Flamisell	46	35.0	8.40	0.744	0.769	0.300	2	18.7 (15.8 – 22.2)	Yes
N. Ribagorzana	**Conangles (CO)**	Ribagorçana	45	20.5	5.52	0.457	0.484	0.045	1	31.0 (12.8 – 149.1)	Yes
	Cavallers (CV)	Noguera Tor	55	34.5	5.18	0.513	0.536	0.020	1	47.0 (27.3 – 101.8)	Yes
	Nicolau (NI)	St. Nicolau	43	13.5	5.56	0.530	0.555	0.040	1	107.4 (35.6 – α)	No
	Erta (ERT)	Erta	72	14.9	3.58	0.431	0.455	0.003	1	66.7 (20.7 – α)	Yes
Ter	**Vallter (TE)**	Ter	65	56.9	3.23	0.379	0.393	0.002	1	61.2 (24.0 – 877.7)	Yes
	Núria (NU)	Freser	155	10.1	4.67	0.489	0.529	0.016	2	53.0 (27.0 – 118.5)	Yes
Llobregat	Riutort (RT)	Llobregat	56	29.1	6.22	0.623	0.630	0.300	2	30.6 (19.2 – 55.3)	No
Hatchery	HAT1	Foreign stock	96	-	7.78	0.666	0.682	-	1	66.8 (52.7 – 87.4)	-

Table 2. Pairwise genetic differentiation (F_{ST} values, Weir and Cockerham, 1984) between brown trout captured in study locations and the current foreign stock. Location code as in Table 1. *$P < 0.05$*

	PL	ER	TOR	AC	MY	FI	CO	CV	NI	ERT	TE	NU	RT
ER	0.003												
TOR	0.192*	0.175*											
AC	0.032*	0.025*	0.183*										
MY	0.190*	0.187*	0.329*	0.171*									
FI	0.121*	0.122*	0.266*	0.108*	0.158*								
CO	0.294*	0.281*	0.425*	0.253*	0.285*	0.247*							
CV	0.193*	0.202*	0.273*	0.226*	0.312*	0.204*	0.334*						
NI	0.203*	0.198*	0.392*	0.221*	0.250*	0.188*	0.331*	0.275*					
ERT	0.299*	0.324*	0.463*	0.310*	0.186*	0.264*	0.454*	0.405*	0.390*				
TE	0.298*	0.323*	0.419*	0.335*	0.379*	0.348*	0.475*	0.379*	0.437*	0.421*			
NU	0.217*	0.199*	0.308*	0.206*	0.291*	0.261*	0.378*	0.329*	0.319*	0.409*	0.354*		
RT	0.260*	0.264*	0.397*	0.259*	0.305*	0.173*	0.364*	0.329*	0.344*	0.288*	0.391*	0.353*	
Foreign stock	0.275*	0.278*	0.390*	0.261*	0.309*	0.116*	0.317*	0.297*	0.298*	0.377*	0.426*	0.366*	0.190*

Table 3. Gene diversity analyses and population structure in the study region using current genotypes, or simulated after 100 generations in scenarios without (Drift), and with supplementation by native stocks, or foreign stock. A_R: Average allelic richness. Total gene diversity (H_T) was partitioned in diversity within locations (H_S) and differentiation between locations (G_{ST}). The per cent of population differentiation assigned to divergence among samples within (*SC*), and among (*CT*) hydrological management units was estimated according to AMOVA analyses. N: effective population size used in simulations; t: indicated accumulated simulated generations. * Significant ($P < 0.05$) comparison relative to generation 0 (G0) values in gene diversity analysis.

		Gene diversity			Population structure	
	A_R	H_T	H_S	G_{ST}	SC (%)	CT (%)
CURRENT						
All populations	5.59	0.787	0.566	0.281	86.14	13.86
Pallaresa basin	6.45	0.745	0.629	0.160	76.65	23.35
Ribagorzana basin	4.96	0.800	0.508	0.376	64.95	35.05
Only stock sources	5.16	0.797	0.513	0.357	-	-
SIMULATIONS						
G0	5.64	0.787	0.560	0.289	88,94	11.06
DRIFT						
N=50, t=100	1.59*	0.778	0.196*	0.749*	99.99	0.01
N=100, t=100	2.31*	0.785	0.348*	0.564*	93.67	6.33
N=1000, t=100	4.27*	0.789	0.521	0.340	87.08	12.92
NATIVE STOCKS						
N=50, t=100	3.23*	0.735	0.415*	0.435*	55.55	44.44
N=100, t=10	5.23	0.787	0.560	0.289	86.58	13.41
t=25	4.83	0.773	0.554	0.283	75.16	24.84
t=50	4.58*	0.769	0.541	0.297	64.50	35.49
t=75	4.33*	0.767	0.532	0.306	60.38	39.62
t=100	3.96*	0.760	0.506	0.334	57.86	42.14
N=1000, t=100	5.51	0.758	0.599	0.209	21.53	78.47
FOREIGN STOCK						
N=50, t=100	4.05*	0.779	0.533	0.315	98.95	1.05
N=100, t=10	6.41	0.808	0.593	0.267	92.11	7.89
t=25	6.08	0.813	0.612	0.247	92.01	7.99
t=100	5.32	0.782	0.623	0.216	97.06	2.94
N=1000, t=100	7.75*	0.794	0.747*	0.060*	100.00	0.00

Table 4. Diversity levels in each location and stock at simulated Generations 0 and 100, in scenarios without (drift), and with supplementation by native stocks or by a foreign stock. H_E: expected heterozygosis (A_R, Average allele richness). F_{ST} : estimated genetic divergence between simulated Generation 0 and 100 at each location and stock. In bold, source location for each simulated native stocks. Effective population sizes of 100 fish for wild locations and 1000 fish for stocks. *$P < 0.05$.

		H_E (A_R)			F_{ST}		
			GEN 100			**0-100**	
	GEN 0	**Drift**	**Native**	**Foreign**	**Drift**	**Native**	**Foreign**
Noguera Pallaresa							
Palomera	0.654 (6.67)	0.312 (2.44)	0.565 (4.44)	0.606 (5.44)	0.223*	0.136*	0.275*
Ermita	0.646 (6.56)	0.402 (2.78)	0.534 (4.22)	0.656 (5.67)	0.170*	0.081*	0.229*
Tor	0.413 (4.67)	0.299 (1.78)	0.471 (4.00)	0.618 (5.44)	0.226*	0.185*	0.267*
Ainet	0.642 (7.78)	0.480 (2.67)	0.447 (4.33)	0.629 (5.44)	0.220*	0.129*	0.146*
Manyanet	0.591(5.00)	0.382 (2.67)	0.598 (5.22)	0.623 (5.44)	0.116*	0.132*	0.236*
Filià	0.761 (8.22)	0.378 (2.89)	0.592 (5.00)	0.597 (5.33)	0.230*	0.135*	0.142*
Noguera Ribagorzana							
Conangles	0.468 (5.11)	0.269 (2.00)	0.434 (3.56)	0.641 (5.33)	0.140*	0.057*	0.257*
Cavallers	0.541 (5.22)	0.394 (2.33)	0.549 (4.22)	0.653 (5.67)	0.164*	0.149*	0.151*
Nicolau	0.558 (5.56)	0.261 (2.00)	0.519 (3.89)	0.588 (5.33)	0.220*	0.295*	0.265*
Erta	0.451 (3.78)	0.307 (1.78)	0.541 (3.78)	0.574 (4.56)	0.203*	0.277*	0.251*
Coastal Basins							
Vallter	0.392 (3.00)	0.225 (1.67)	0.284 (2.67)	0.667 (5.44)	0.192*	0.140*	0.249*
Nuria	0.537 (5.67)	0.463 (2.67)	0.552 (2.89)	0.587 (5.11)	0.202*	0.166*	0.256*
Riutort	0.629 (6.11)	0.349 (2.44)	0.496 (3.22)	0.665 (4.89)	0.181*	0.253*	0.163*
Simulated Stocks							
Pallaresa basin	0.653 (6.78)	-	0.631 (5.50)	-	-	0.024*	-
Ribagorzana basin	0.480 (5.56)	-	0.443 (3.78)	-	-	0.045*	-
Coastal basins	0.392 (3.33)	-	0.355 (3.00)	-	-	0.021*	-
Foreign stock	0.679 (7.78)	-	-	0.638 (6.22)	-	-	0.031*

Table 5. Estimate ancestries in each populations after 100 generations in scenarios without (Drift) and with supplementation with native stocks, and Ne of 100 fish. Np: average remaining ancestry of the local gene pool; Ss: average ancestry of the simulated native stock. In each basin, location in bold was the source of the native stock.

| Basin/Location	Drift		Supplementation	
	Np	Ss	Np	Ss
Noguera Pallaresa				
Palomera	0.983	0.957	0.890	0.934
Ermita	0.976	0.957	0.975	0.953
Tor	0.950	0.867	0.778	0.926
Ainet	0.795	0.675	0.862	0.961
Manyanet	0.966	0.580	0.653	0.781
Filià	0.966	0.197	0.845	0.675
Noguera Ribagorzana				
Conangles	0.993	0.927	0.981	0.905
Cavallers	0.895	0.407	0.675	0.635
Nicolau	0.935	0.227	0.431	0.571
Erta	0.956	0.131	0.397	0.512
Coastal basins				
Vallter	0.989	0.961	0.986	0.959
Nuria	0.911	0.624	0.927	0.705
Riutort	0.940	0.127	0.429	0.668
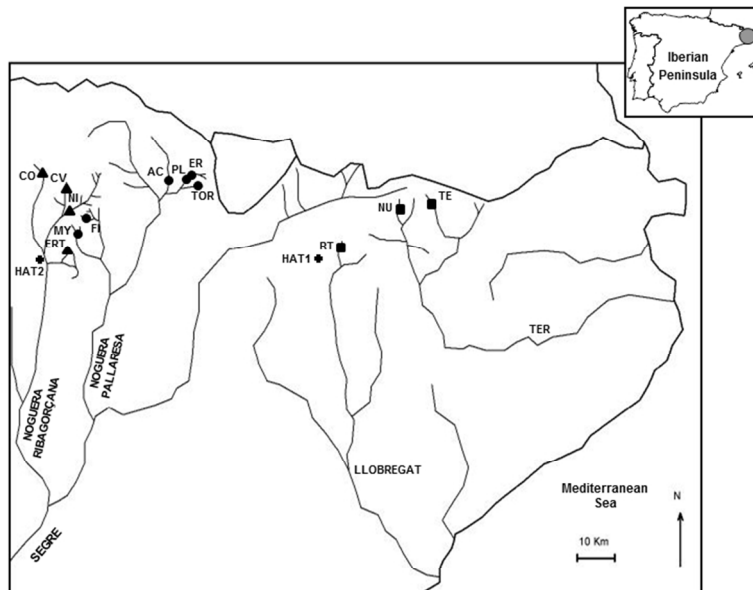
Fig. 1. Geographic locations of the brown trout collections and the two hatcheries (HAT) in the study region. Location codes are defined in Table 1.
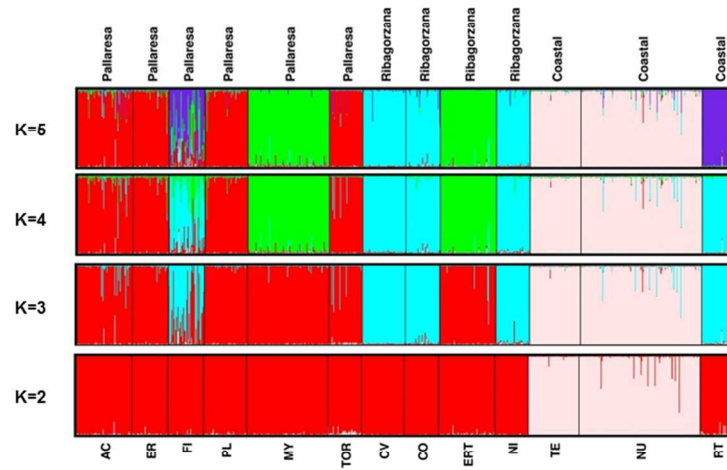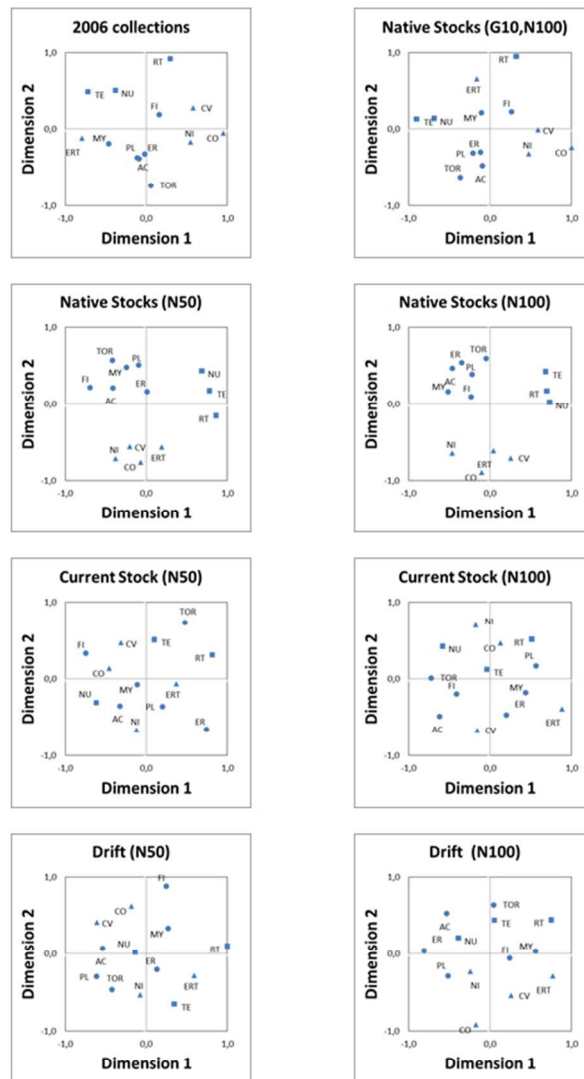254x190mm (96 x 96 DPI)

Fig. 2. Current individual sample relationships indicated by STRUCTURE analysis considering 2, 3, 4 and 5 genetic groups. Each individual is represented by a vertical bar partitioned into segments according to the proportion of the genome assigned to each of the identified clusters. Location codes are defined in Table 1. 254x190mm (96 x 96 DPI)

Multidimensional scaling (MDS) plots of population genetic structure from captured collections (2006 collections), and from simulations without (drift), and with supplementation by current exogenous or native stocks. Squares represent Coastal management unit, circles Noguera Pallaresa, and Noguera Ribagorçana is represented by triangles. Locality codes are defined in Table 1.

190x254mm (96 x 96 DPI)