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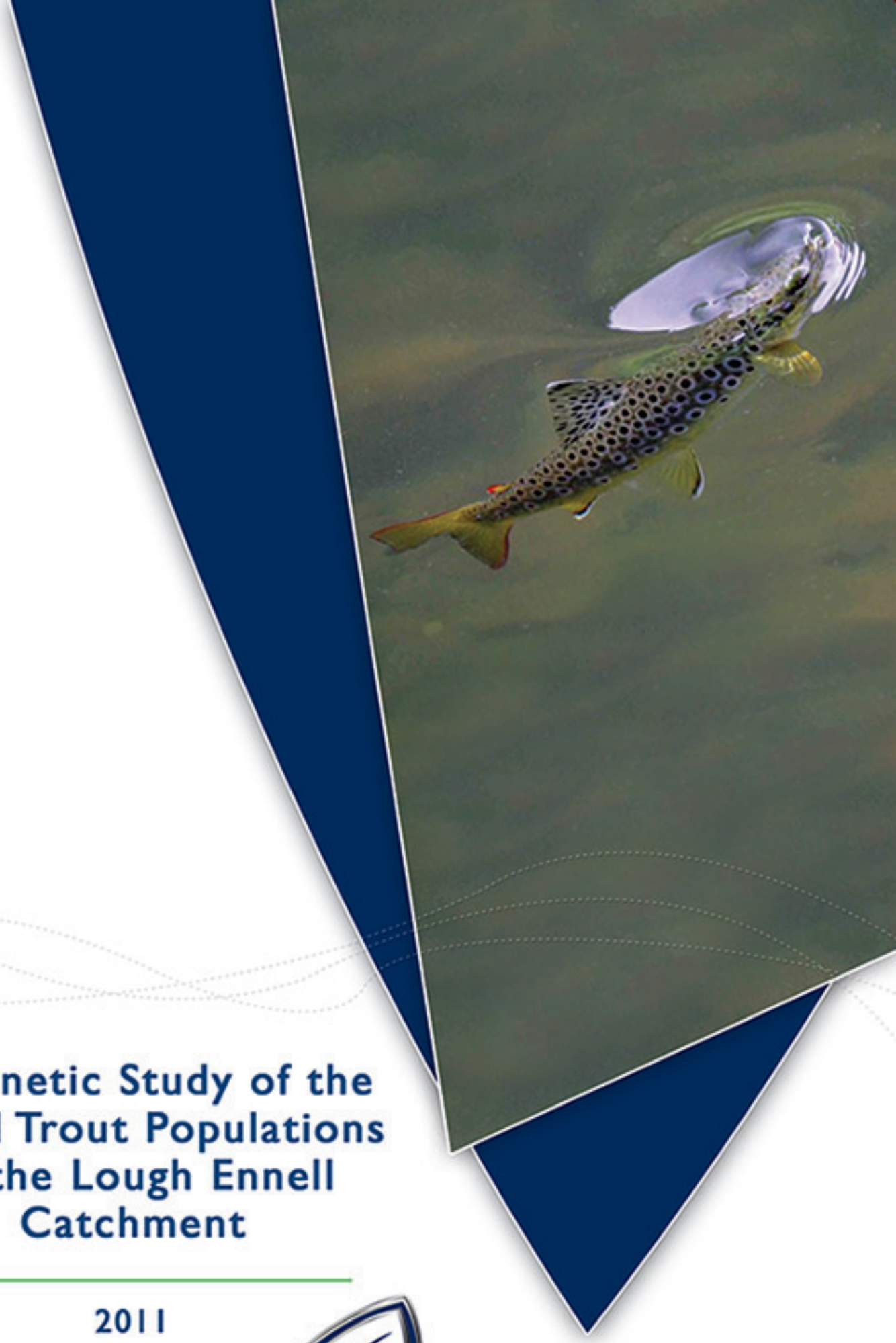

A Genetic Study of the Mixed Trout Populations of the Lough Ennell Catchment

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Environmental River Enhancement Programme

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TECHNICAL REPORT FOR INLAND FISHERIES IRELAND

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A genetic study of the mixed trout populations of the Lough Ennell catchment

Technical Report for Inland Fisheries Ireland

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1 Background

Salmonid fish are an integral part of the post-glacial fish fauna of temperate inland waters and have constituted an important resource for human societies across the centuries (Youngson et al. 2003). Brown trout (*Salmo trutta* L.) is an ecologically diverse species, whose natural geographical distribution ranges from Norway to North Africa and from Iceland to Afghanistan (Elliott 1994).

Brown trout has a considerable socioeconomic value because it represents an important resource in many countries for commercial and sport fisheries (Laikre et al. 1999). In Ireland, in particular, brown trout is one of the most popular species for sport fisheries, and as such supports a large tourist industry (Willams & Ryan 2003), especially in rural areas.

Brown trout population dynamics are very sensitive to the alterations of the physical and natural environment (Elliott 1994) associated with anthropogenic impacts such as arterial drainage, cultural eutrophication, introduction of alien species and stocking of domesticated fishes; all of which may alter the demographic and ecological equilibria of these populations.

Lough Ennell is an established trout fishery since the 19th century. The catchment comprises eight main rivers that drain directly into the lake and provide breeding grounds for brown trout populations. In the 1970s the streams were subject to an arterial drainage programme to improve agricultural productivity (Gargan et al. 2002) with consequent disruption of the riverine habitat suitable for brown trout populations and the inevitably negative impact on the lake fishery. In the mid 1970s the impoverished trout populations experienced a collapse due to substantial eutrophication of the lake water, caused by municipal waste discharge (John et al. 1982; Champ 1998) that favoured the establishment of roach populations, introduced at the end of the 1970s (Anon 2004).

To enhance the trout populations in the catchment domesticated fish, bred in the Roscrea fish of farm, were stocked in the main rivers flowing in to Lough Ennell. Artificial stocking is a widely practiced activity by fishery management in order to mitigate the negative impacts of environmental degradation (Youngson et al. 2003), the actual effects of this practice very often affects negatively the wild populations causing the loss of genetic variability and local

adaptations as consequence of interbreeding between the wild and the domesticated strain (Laikre et al. 1999 and refernces therein).

The use of genetic markers has been effective to assess the long-term genetic impact of domesticated fish on wild populations (Wollebaek et al. ; Hansen et al. 2001) and the levels and distribution of genetic diversity (VanDehey et al. 2009). Genetic data have also been used to effectively estimate the stock composition of mixed fisheries (Koljonen & Wilmot 2005; Ruzzante et al. 2006; Bekkevold et al. 2007; Beacham et al. 2009) with microsatellites still representing the most reliable and powerful tool.

The aim of the present study is to estimate the genetic impact of domesticated fish on wild brown trout populations in the Ennell catchment and estimate the proportional contribution of the trout spawning components to the lake fishery. The information obtained is expected to contribute significantly to the implementation of effective, evidence-based management strategies, with a view to guaranteeing sustainability of one of the most important trout fishery in Ireland.

2 Population Structure and impact of fish stocking

2.1 Materials and methods

2.1.1 Study area and Sample collection

The Lough Ennell catchment presents a complex network of rivers most of which contains spawning and nursery areas suitable for brown trout.

The five major rivers flowing into Lough Ennell have been selected for the present study to examine brown trout genetic population structuring and to evaluate the genetic contribution of stocked fish from Roscrea fish farm in the catchment (Table 2.1, Fig. 2.1a).

Samples for molecular analysis were collected from the Ennell streams by electrofishing brown trout parr (1+ year class). Fish were sampled using a portable generator with a unit control either from the bank (in shallow stream) or from a boat (in larger streams). A total of 50 individuals were collected in each stream, at least three repeated fishing were carried out at a distance of at least 100m from one another in order to ensure representative sampling of different family groups (Allendorf & Phelps 1981; Hansen et al. 1997). An additional sample of 50 individuals was collected from Roscrea fish farm.

2.1.2 Microsatellite data

DNA was isolated from fin clip tissue which had been stored in absolute ethanol, using a modified salt/chloroform extraction protocol (Miller et al. 1988) that included an additional chloroform/isoamyl alcohol (24/1) step after adding the saturated NaCl solution (Petit et al. 1999).

A total of 244 individuals were (PCR) amplified and genotyped at 12 polymorphic (one tetranucleotide and 11 dinucleotide) microsatellite loci: BS131, T3-13 (Estoup et al. 1998), Str15, Str60, Str73 (Estoup et al. 1993), Ssa197, Ssa85 (O'Reilly et al. 1996), Str85, Str543, Str591 (Presa & Guyomard 1996), OmyFgt1TUF (Sakamoto et al. 1994; Petit et al. 1999), SSOSL417 (Slettan et al. 1995). Fragments were amplified in two 10µl multiplex PCR reactions containing 1µL of DNA extract and 1×Multiplex PCR MasterMix (QIAGEN) and labeled primers (FAM, VIC, PET and NED Applied Biosystems©) with concentrations ranging from 0.1 to 0.30µl. Amplification conditions were as follows: 95 °C for 15 min; 35 cycles of 94 °C for 45 s, 55 °C for 45 s, 72 °C for 45 s and a final extension at 72 °C for 45 min. All PCR products were run on a 16-capillary system ABI 3130xl Genetic Analyzer (Applied Biosystems©) and sized alongside an internal lane standard (600 LIZ; Applied Biosystems©) using the program GeneMapper version 4.0 (Applied Biosystems©).

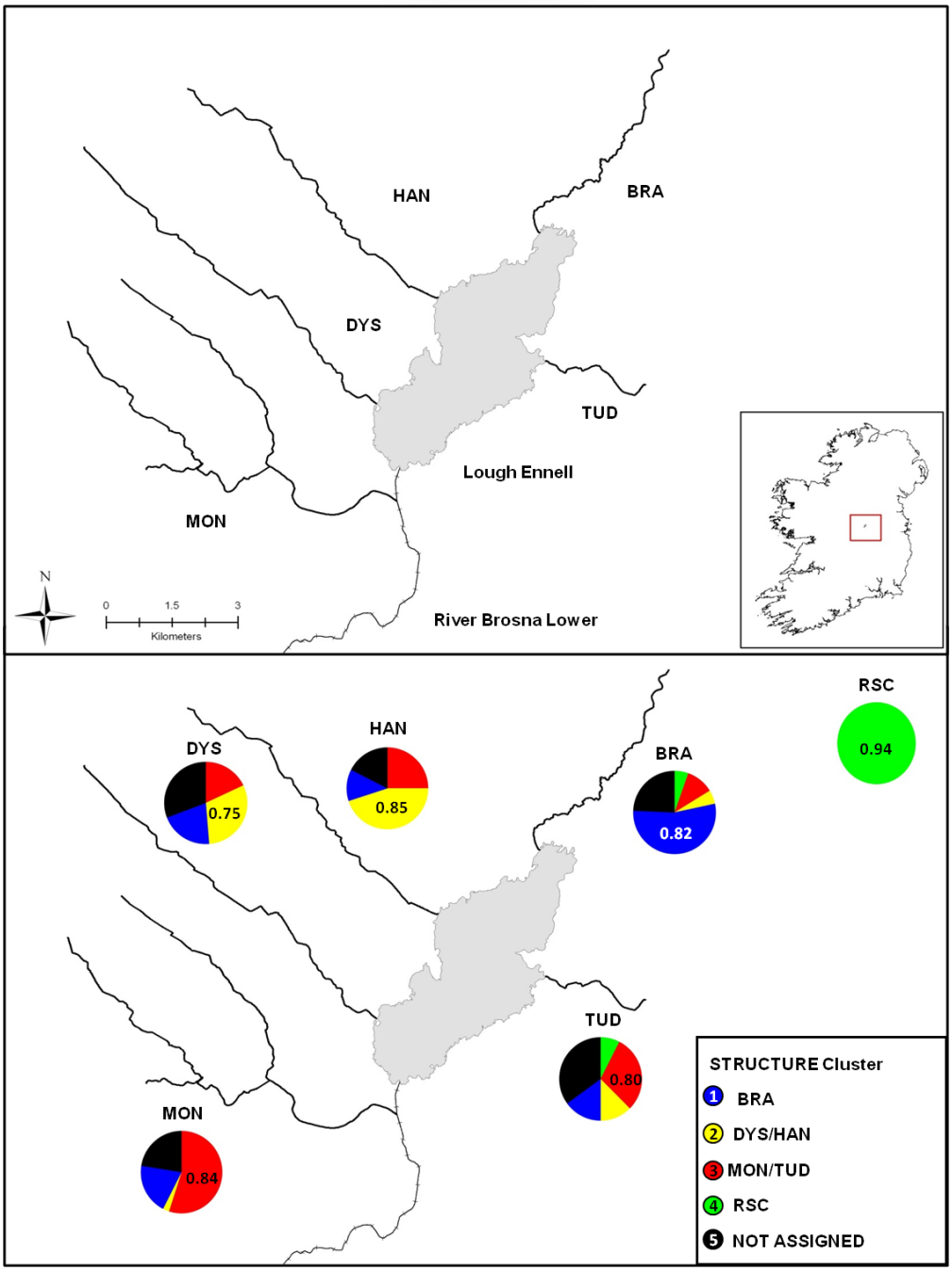


Figure 2.1 a) Inset: location of the Ennell catchment in Ireland. Main figure: the streams where brown trout were sampled. BRA, Brosna Upper; DYS, Dysart; HAN, Hanstown; MON, Monaghanstown; TUD, Tudenhan.

b) Geographical distribution of genetic clusters identified by Structure. Cluster 1 (blue) includes brown trout from the river Brosna Upper; Cluster 2 (yellow) is found in the Dysart and Hanstown rivers; Cluster 3 (red) is present in Monaghanstown and Tudenhan rivers; Cluster 4 (green) includes only individuals from Roscrea fish farm. Black corresponds to individuals that could not be assigned with confidence to any of the known populations.

2.1.3 Data analysis

The software Micro-Checker (van Oosterhout et al. 2004) was used to check for the presence of null alleles, large allele drop-out and possible scoring errors in each population sample.

To assess the genetic variation within and among the studied populations, expected unbiased (H_E) (Nei 1978) and observed (H_o) heterozygosities were calculated using the program Fstat 2.9.3 (Goudet 1995), which was also used to estimate allelic richness (A_R) using the rarefaction method (El Mousadik & Petit 1996) and overall and pairwise F_{ST} values θ-statistics from (Weir & Cockerham 1984). Significance was tested by randomizing multi-locus genotypes between pairs of populations. Fstat 2.9.3 was also used to estimate the coefficient of inbreeding, F_{IS}, for each locus in each population sample, with significance levels calculated by randomizing alleles among individuals 10,000 times, hence determining deviations from Hardy-Weinberg equilibrium (HWE). Significance values were adjusted for multiple comparisons using a sequential Bonferroni method (Rice 1989). Pairwise F_{ST} values were visualised using multidimensional scaling analysis (MDSA) as implemented in the software XLstat7.5 (Addinsoft ™).

The Bayesian clustering approach implemented in the software STRUCTURE 2.3.3 (Pritchard et al. 2000) was used to infer the most likely number of population clusters (K) constituting the sample, assigning individuals with similar multilocus genotypes to the same group, in order to minimise Hardy-Weinberg and linkage disequilibria within clusters. Each individual is then assigned an admixture coefficient (*Q*) that estimates the proportions of an individual's genome derived from each inferred cluster and sums up to 1. For instance, if an individual has a 0.95 *Q*-value for one cluster and 0.05 from other clusters, that fish has a high probability of being a „genetically pure" individual belonging to the first cluster. *Q*-values split between clusters signify a „mixed" genetic identity. Each individual was assigned to one of the identified clusters if its highest Q value was at least twice as high as the second highest value for another cluster. Only individuals with complete data for at least eight loci (N=244) were included in the computation with Structure.

The number of private alleles was estimated to gather information on gene flow levels (Slatkin 1985). A private allele is one found only in one population (Allendorf and Luikart 2007) and its average frequency depends on mutation events and the migration rate. Generally, the number of alleles that are private in a population is low when gene flow is high. Additionally, BAYESASS 1.3 (Wilson & Rannala 2003) was employed to test whether overall patterns of migration within the catchment were consistent with the results obtained using other methods. Default settings of 3,000,000 iterations (with 1,000,000 burn-in) were used, with delta values allowing between 40 and 60% of parameter changes across iterations. Only migration estimates whose confidence intervals did not overlap with the confidence limits of a simulated distribution with no information content (which for five populations are between $1.79 \cdot 10^{-05}$ and 0.185) were considered reliable (see BAYESASS manual).

Effective population sizes were estimated using the method based on gametic disequilibrium as implemented in the software LDNe 3.1 (Waples & Do 2008), which requires only a single

sample from each study population. Particularly, when the test is conducted on individuals of the same age class, the program effectively estimates the effective number of breeders (N_b) for a given year.

2.2 Results

All the 12 loci analyzed showed no evidence of null alleles in the five populations sampled. The microsatellites used in this study were highly polymorphic, with 3 (STR591) to 33 (OmyFgt1TUF) alleles per locus and an expected heterozygosity across populations ranging from 0.57 to 0.69 (Table 2.1). Levels of genetic diversity (A_R and H_E) were equally distributed across the populations examined, except for MON, which displayed lower diversity (Table 2.1). Overall F_{ST} value was high and significant (0.064), indicating strong genetic structuring among the study populations (Table 2.2). The genetic variance was not homogenous among population samples: in fact, RSC showed the highest pairwise values (F_{ST} ranged from 0.103 to 0.147) against all other populations, among which instead F_{ST} ranged from -0.001 to 0.030. When the RSC sample was removed, the overall genetic differentiation among populations decreased (F_{ST}=0.018). All F_{ST} pairwise comparisons were significant prior correction to multiple tests, except for the pair DYS vs. HAN. After sequential Bonferroni correction, all F_{ST} pairwise comparisons were significant (Table 2.2; Fig. 2.2).

Structure results suggested K=4 as the most likely number of clusters (Fig. 2.1b, 2.3). Cluster 1 is constituted by individuals from the Brosna Upper River. The majority of individuals in cluster 2 (79%) come from two eastern rivers of the catchment (DYS and HAN); cluster 3 is mostly represented by the brown trout in the Monghanstown and Tudenhan river; Cluster 4 almost entirely (91%) corresponds to trout sample from Roscrea fish farm (Fig. 2.1b). Around 30% of the individuals from DYS and TUD could not be assigned with confidence to any of the four clusters identified, whereas in the other populations the proportion of not assigned individuals was around 20% (Fig. 2.1b). The population that had the highest average membership coefficient to any one cluster (>90%) was RSC (cluster 4) followed by MON (94%, Cluster 3) (Fig. 2.1b). Trout from DYS and TUD rivers proved to be less genetically „pure", with a greater proportion of unassigned individuals (>31%) and membership *Q*-values below 0.8 (Fig. 2.1b).

Migration rate estimates produced by BAYESASS proved fairly congruent with other lines of evidence. In this instance the fish farm sample (RSC) was excluded from the analysis. Within the catchment, DYS appears to be the main source of migrants, seeding on the eastern sub-catchment both HAN and MON, contributing with 31% (95% C.I. 0.266–0.329) and 26% (95% C.I. 0.085–0.326) of immigrants respectively. On the western side, BRA receives 27% (95% C.I. 0.201–0.320) and TUD 0.30% (95% C.I. 0.236–0.328) immigration from DYS. No other migration estimates were significantly different from a random distribution.
Estimates of effective population size, N_e, indicate that populations from the rivers Hanstown and Tudenhan are particularly small (Table 2.1)

Table 2.1 Sampling location, sample code and sample size (N) for the trout populations in the study. Genetic diversity indices averaged over loci include: H_E, expected heterozigosity; H_O, observed heterozigosity; N_A, mean number of alleles; A_R, allelic richness; A_P, number of private alleles; $F_{IS,}$ coefficient of inbreeding; estimated effective population size (N_e), with associated 95% confidence intervals.

Sampling location	**Sample Code**	**N**	H_E	H_O	N_A	A_R	A_P	F_{IS}	N_e **(95% CI)**
Brosna Upper	BRA	37	0.64	0.63	7.08	6.48	6	0.006	113.9 (61.9-434.9)
Dysart	DYS	39	0.60	0.62	6.92	6.03	5	-0.018	1540.3 (132.8-∞)
Hanstown	HAN	40	0.58	0.56	6.42	5.78	4	0.045	61.3 (39.4-117.6)
Monaghanstown	MON	40	0.57	0.58	5.83	5.40	1	-0.020	96.3 (53.8-301.6)
Tudhenan	TUD	40	0.58	0.57	6.58	6.01	2	0.027	55.4 (38.1-91.5)
Roscrea	RSC	48	0.69	0.68	7.33	6.64	6	0.007	238.9 (111.0-∞)

Table 2.2 Matrix of F_{ST} pairwise comparisons (below diagonal) and non-adjusted p-values (above diagonal). Bold values are significant after sequential Bonferroni correction (initial α= 0.0033).

	Brosna Upper	Dysart	Hanstown	Monaghanstown	Tudenhan	Roscrea
Brosna Upper		0.00007	0.00007	0.00007	0.00007	0.00007
Dysart	**0.0190***		0.56773	0.00007	0.00080	0.00007
Hanstown	**0.0303***	-0.0005		0.00007	0.00020	0.00007
Monaghanstown	**0.0290***	**0.0117***	**0.0225***		0.00007	0.00007
Tudenhan	**0.0302***	**0.0083***	**0.0120***	**0.0199***		0.00007
Roscrea	**0.1030***	**0.1244***	**0.1378***	**0.1469***	**0.1255***	

***** Significance at 0.05 level

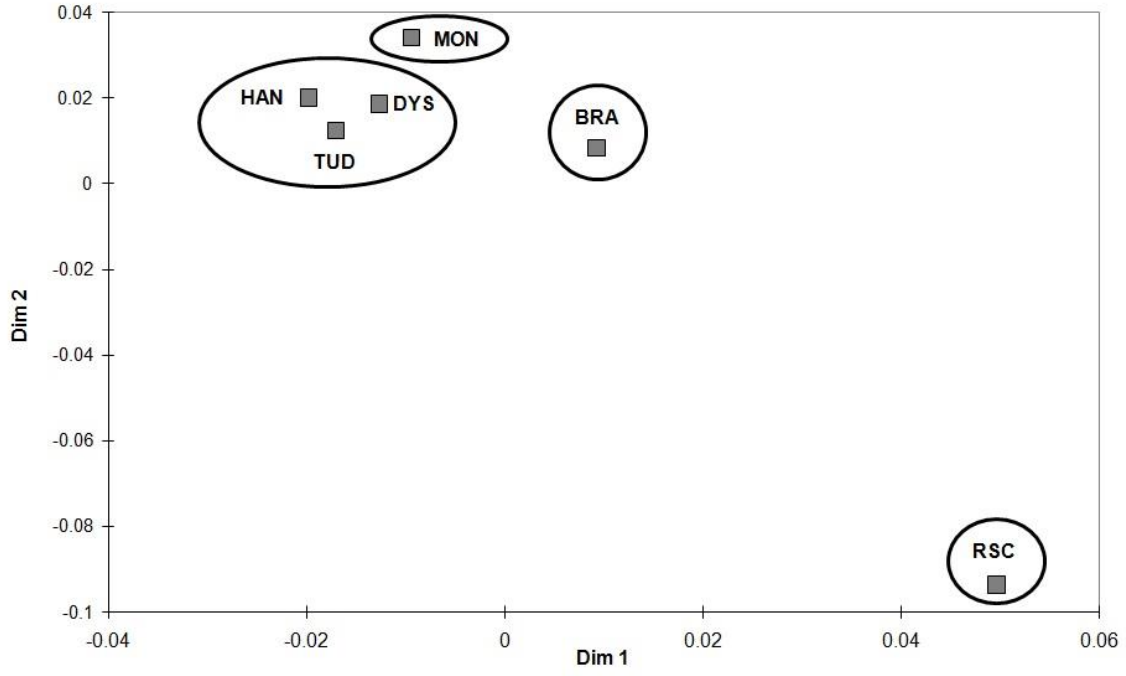


Figure 2.2 Multidimensional scaling plot based on the matrix of F_{ST} pairwise comparisons. The ellipses represent the groups identified by the AMOVA.

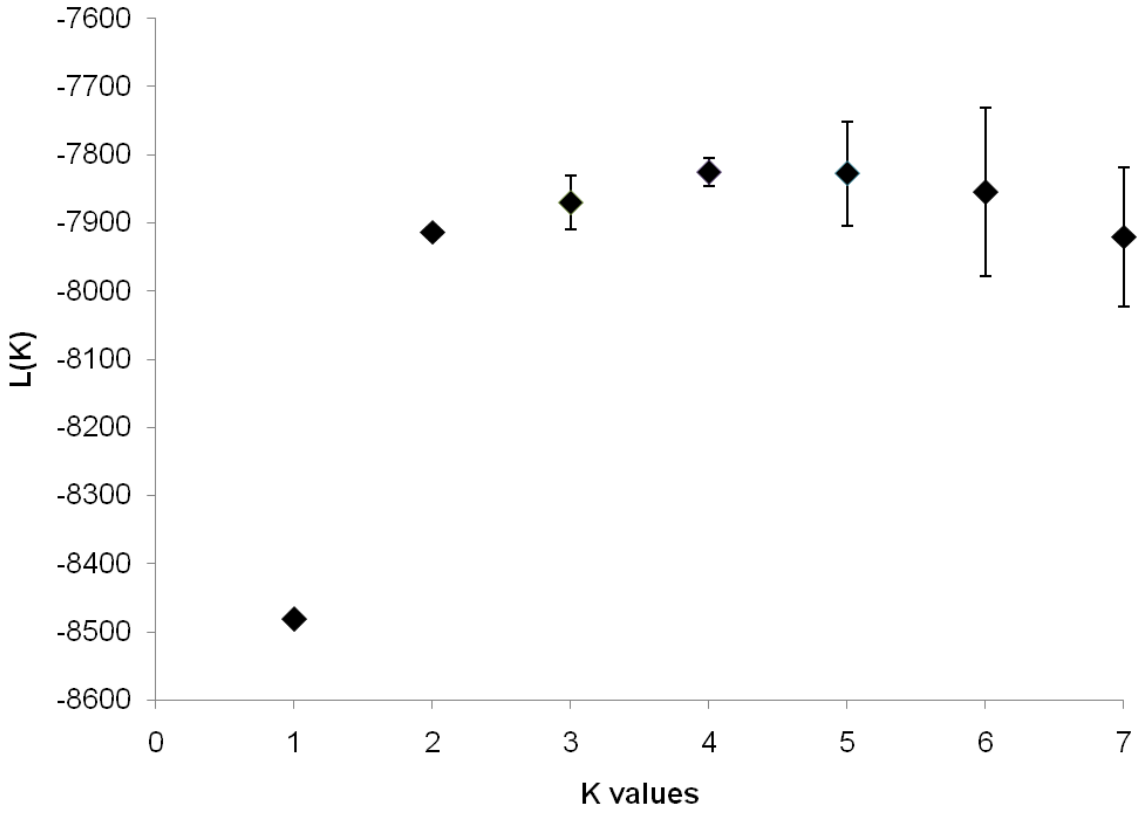


Figure 2.3 Mean L(K) (± SD) over 5 runs for each K value explored using STRUCTURE showing the lowest variance for K= 4.

3 Lake Stock

3.1 Materials and methods

3.1.1 Study area and sample collection

Mixed fishery data were collected from adult lake trout obtained by either gill netting or angling in 2004 (N= 55), in 2006 (N=38) and 2007 (N= 23) (Table 3.1). Tissue samples for molecular analyses were collected by clipping the caudal fin.

DNA isolation, PCR-amplification and genotyping were carried out following identical procedures as indicated in section 2.1.1. Data quality-check, estimates of genetic variability measures and F-statistics, as well as Bayesian cluster analysis followed methods illustrated in section 2.1.3.

3.2 Results

All the 12 loci analyzed showed no evidence of null alleles in the lake fishery samples. The microsatellites used in this study were highly polymorphic, with 3 (Str60 and STR73) to 26 (OmyFgt1TUF) alleles per locus. Genetic variability (H_E and A_R) was equally distributed in the mixed stock samples (Table 3.1). F_{ST} pairwise comparisons showed that all mixed stock samples (ENN04, ENN06, ENN07) were identical to one another and were genetically matching the fish from the eastern rivers (DYS and HAN) and Tudenhan River (Fig. 3.1)

STRUCTURE analysis of mixed fishery samples (ENN04, ENN06 and ENN97) and baselines (BRA, DYS, HAN, TUD and RSC) suggested K= 4 as the most likely number of clusters. The genetic clusters identified by STRUCTURE correspond to those described in section 2.2.

The proportions of 2004 mixture individuals assigned to the baselines clusters indicated that a similar proportion of trout was assigned to Cluster 1 (17%), Cluster 2 (15%) and Cluster 3 (13%). In 2006 the assigned proportions of the mixed samples showed a decrease of the individuals from Cluster 1 (7%) and a similar contribution from Cluster 2 (19%) and Cluster 3 (24%). In 2007 there was an increase in the contribution of trout originating from Cluster 1 (30%) and slight changes in the proportion of trout assigned to Cluster 2 (22%) and Cluster 3 (17%) (Table 3.2; Fig. 3.2). The contribution of Cluster 4 (RSC) to the lake fishery was negligible in all samples. The samples not assigned to any of the baseline clusters were estimated around 50% in ENN04 and ENN06 and decreased to 30% in ENN07 (Table 3.2 and Fig. 3.2).

Table 3.1 Sampling location, sample code and sample size (N) for the samples used in the analysis. Genetic diversity indices averaged over loci include: H_E expected heterozigosity; H_O observed heterozigosity, N_A mean number of alleles and A_R allelic richness. For the river baselines values see Table 2.1.

Sample Name	Sample Code	N	H_E	Ho	N_A	A_R
Ennell 2004	ENN04	55	0.57	0.55	7.17	5.47
Ennell 2006	ENN06	38	0.56	0.54	6.42	5.37
Ennell 2007	ENN07	23	0.57	0.55	5.50	5.31

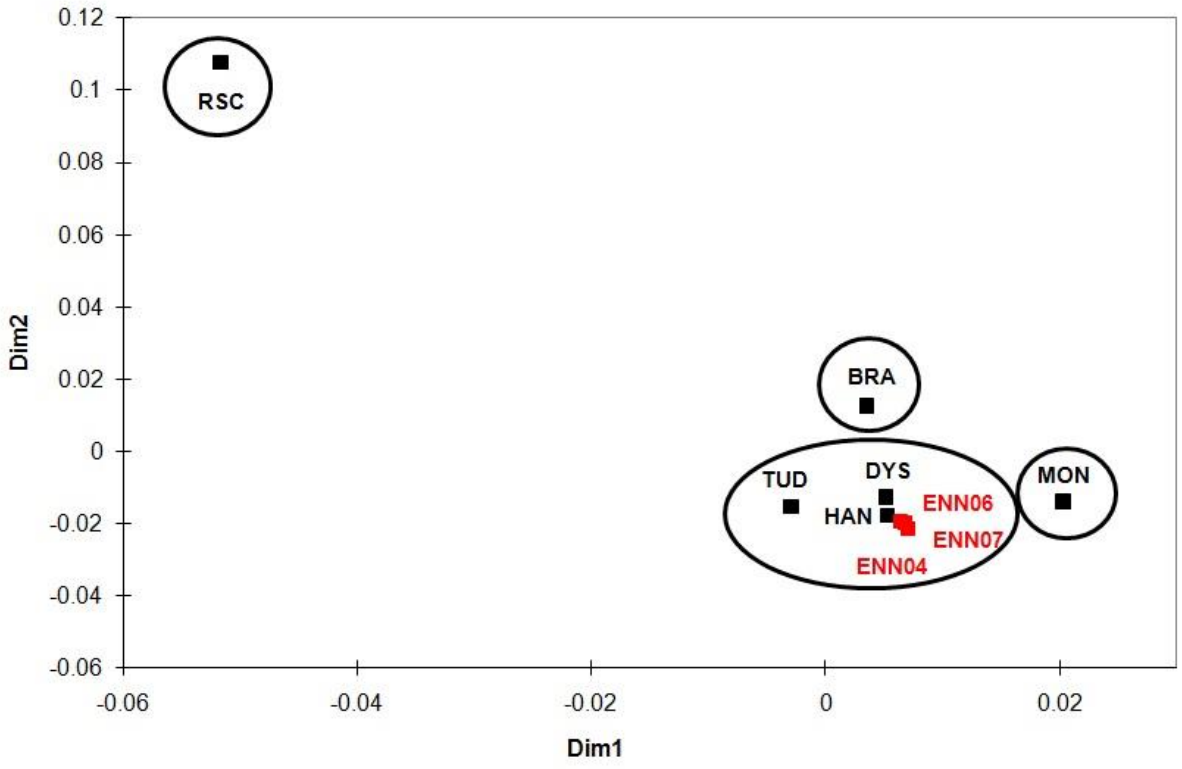
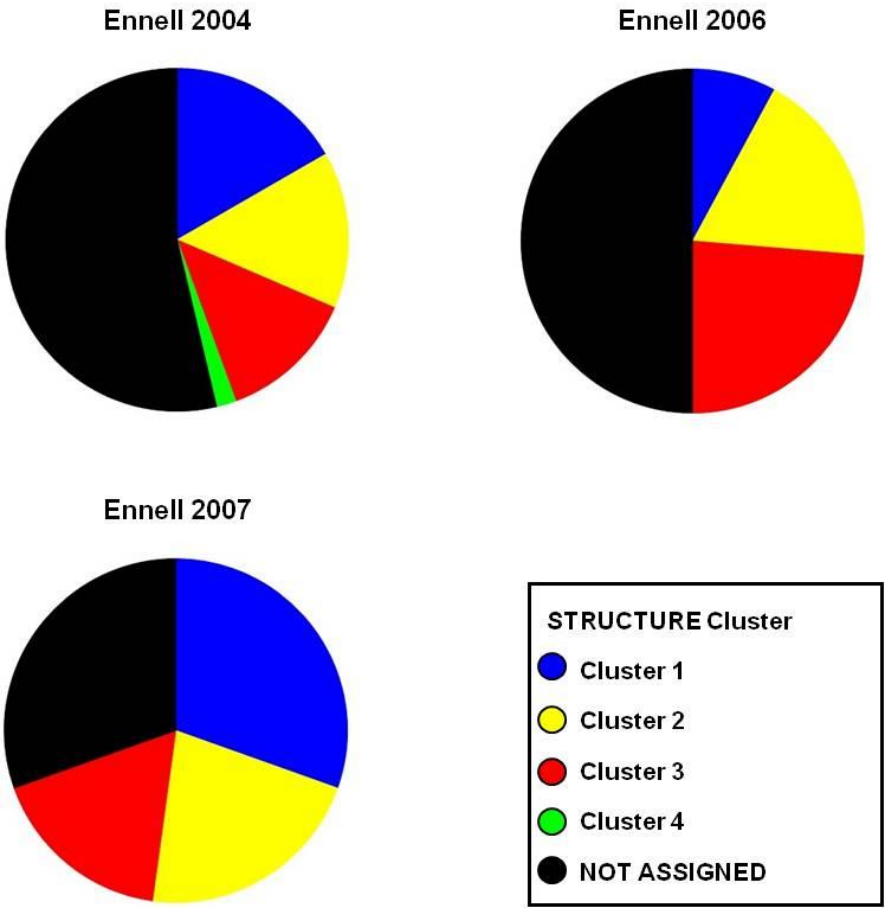


Figure 3.1 Multidimensional scaling plot based on the matrix of F_{ST} pairwise comparison

Table 3.2 Proportions of lake individuals assigned to the genetic clusters of baseline populations identified by STRUCTURE. Proportions of samples not assigned to any baseline clusters are reported as well. Mixture individuals comprise mixed-stock samples collected in 2004, 2006 and 2007.Trout populations in Brosna Upper river form cluster 1, cluster 2 includes the brown trout from the Dysart and Hanstown rivers and trout populations in Monaghanstown and Tudhenam form cluster 3, cluster 4 consist only of Roscrea fish.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Not Assigned
ENN04	16.67	14.81	12.96	1.85	53.70
ENN06	7.89	18.42	23.68	0.00	50.00
ENN07	30.43	21.74	17.39	0.00	30.43

Figure 3.2 Proportions of mixture individuals assigned to the genetic clusters of baseline populations identified by Structure. Mixture individuals comprise mixed-stock samples collected in 2004, 2006 and 2007. Colours follow those in Fig. 2.1b. Black corresponds to individuals that could not be assigned with confidence to any of the known population



4 Conclusions:

Both F_{ST}-based analysis of genetic variance and Bayesian clustering sorted the population in the Ennell catchment in three main genetically distinct groups. These groups present different level of admixture and, in particular, Dysart and Tudenhan rivers show very "blended genotypes" and high proportion of "unassigned" component ("black" in Fig. 2.1b), probably deriving from multigenerational straying among the rivers. The river Dysart represents the major source of migrants seeding all the other rivers in the catchment.

Individual-based analysis of the mixed-stock samples indicates that the lake fishery samples originate in similar proportions (~ 20% each cluster) from three baseline clusters (Cluster 1, Cluster 2 and Cluster 3; Table 3.2 and Fig. 3.2). The constitution of the mixed lake stock appears temporally stable, apart from the Brosna population which contribution is smaller in 2006. The amount of samples that were not assigned to any of the source populations is high (between 50% and 30%) indicating that either these individuals have a much admixed genotype or they belong to unsampled populations.

The present results illustrates clearly that there is no genetic contribution of stocked, domesticated trout from Roscrea fish farm in both the river populations and the lake fishery in the Ennell catchment.

Hence this study supports the current view that stocking wild populations with domesticated fish is not a suitable choice both under a fisheries and conservation perspective. In fact the artificial stocking contributed very little to rehabilitating the populations in the catchment concurrently increasing the risk for permanent introgression of the domestic strain into the wild gene pool with consequent decrease of genetic diversity and fitness.
Therefore current fishery management and conservation should focus on keep improving environmental conditions of the breeding and nursery areas in the catchment.

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