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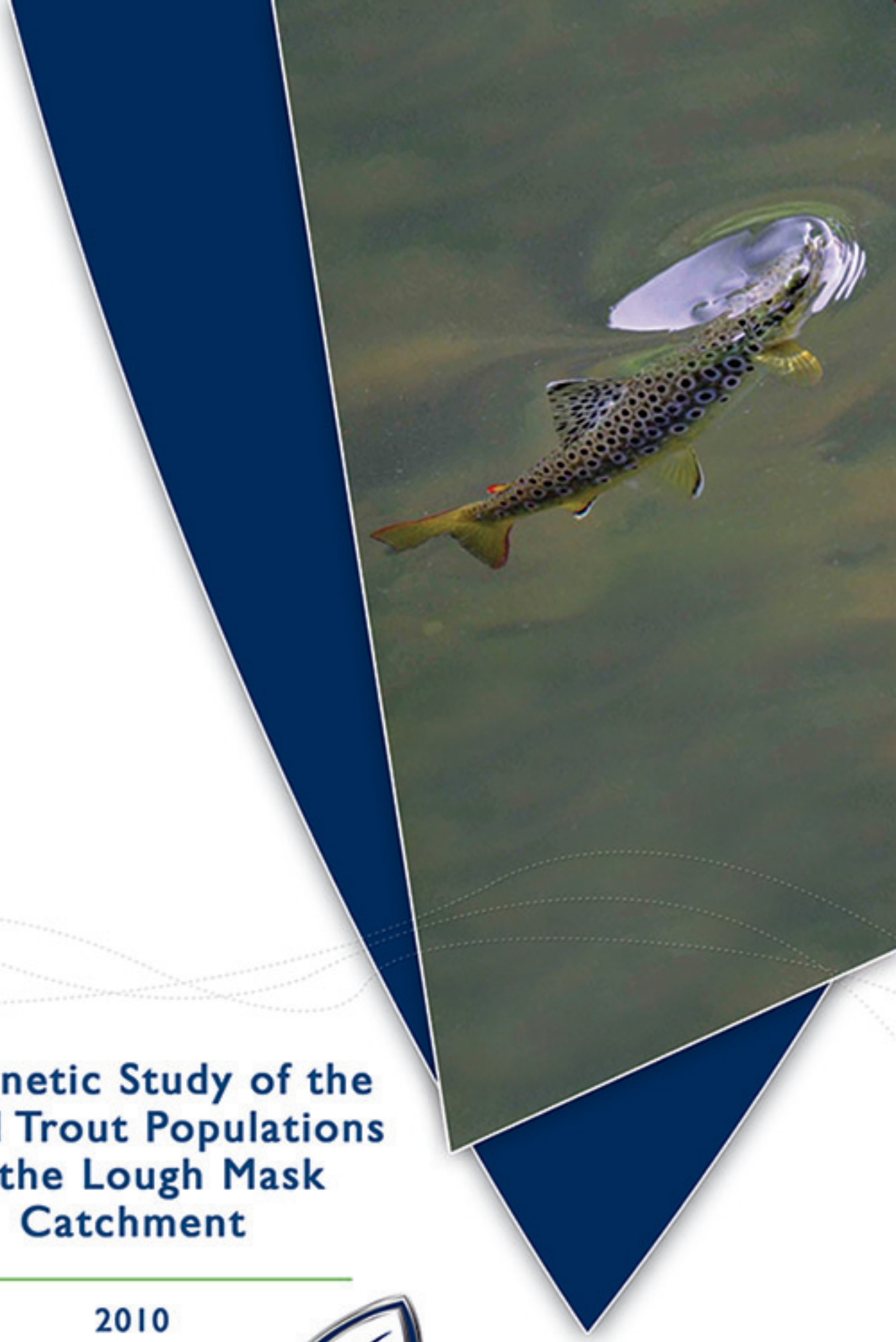

A Genetic Study of the Mixed Trout Populations of the Lough Mask Catchment

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A genetic study of the mixed trout populations of the Lough Mask catchment.

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Alexia Massa-Gallucci and Stefano Mariani

School of Biology & Environmental Science, University College Dublin, Belfield, Dublin 4.

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

Final report for IFI

Stefano Mariani and Alexia Massa-Gallucci

UCD School of Biology & Environmental Science

1 Background

Brown trout (*Salmo trutta* L.), native to Europe, north western Asia and North Africa, is a highly polytypic and ecologically diverse species, which for centuries has played a significant social and economic role in human societies in temperate regions, where it has been extensively exploited by both commercial and sport fisheries.(Youngson et al. 2000). 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 and Ryan 2003), especially in rural areas.

Lough Masks and its inflowing streams, situated in the west of the country (Fig.2.1), is one of Ireland's largest and most important wild salmonid catchments. Over the years, Lough Mask has gained an international reputation as an excellent salmonid fishery, as it is one of the few habitats where anglers have the opportunity to also capture the long-lived, highly-prized, piscivorous 'ferox' eco-phenotype, which is endemic to only a few Irish and Scottish lakes (Ferguson 2004) .

Population diversity, at both ecological and genetic levels, is not only threatened by fisheries, but also by other anthropogenic drivers such as urban development and associated discharges, agricultural nutrient run-off, and introduction of alien species; all of which may alter the demographic and ecological equilibria of these populations. In this scenario it is likely that the more exploited and/or less productive components of the fishery will be removed first, with the consequent loss of diversity (Policansky and Magnuson 1998; Hilborn et al. 2003) which is critical to the resilience and the long-term viability of populations.

Studies using molecular markers are effective in assessing the levels and distribution of genetic diversity and useful as a fundamental step in identifying conservation units (Waples 2006; Beacham et al. 2008; VanDehey et al. 2009). Genetic data have also been used to effectively estimate the stock composition of mixed fisheries (Koljonen and 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 unravel the patterns of neutral genetic diversity in the Mask catchment and estimate the proportional contribution of the trout spawning components to the lake fishery. This study also represents one of several past and ongoing efforts that employ the same approach and set of genetic markers to deliver an up-do-date and directly comparable characterisation of selected brown trout assemblages in the Republic of Ireland. It is hoped that the present results, and their integration within the broader context, can lay the foundations for improved management of the most important trout fisheries in Ireland.

2 Population Structure

The Lough Mask catchment area has a complex network of sub-catchments and rivers, most of which contain spawning and nursery areas for brown trout.

The eight major rivers flowing into Lough Mask have been selected for the present study to represent the overall gene pool of brown trout in this catchment, with the inclusion of a landlocked sample obtained from the isolated stream Aille, which harbours an isolated brown trout population at the west of the catchment (Fig. 2.1a, Table 2.1).

2.1 Materials and method

2.1.1 Study area and sample collection

Samples for molecular analyses were obtained by clipping the caudal fin of brown trout parr (1+ year class) collected by electrofishing. 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 and Phelps 1981; Hansen et al. 1997).

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 430 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 and 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 the same as in Massa-Gallucci et al (2010). 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©).

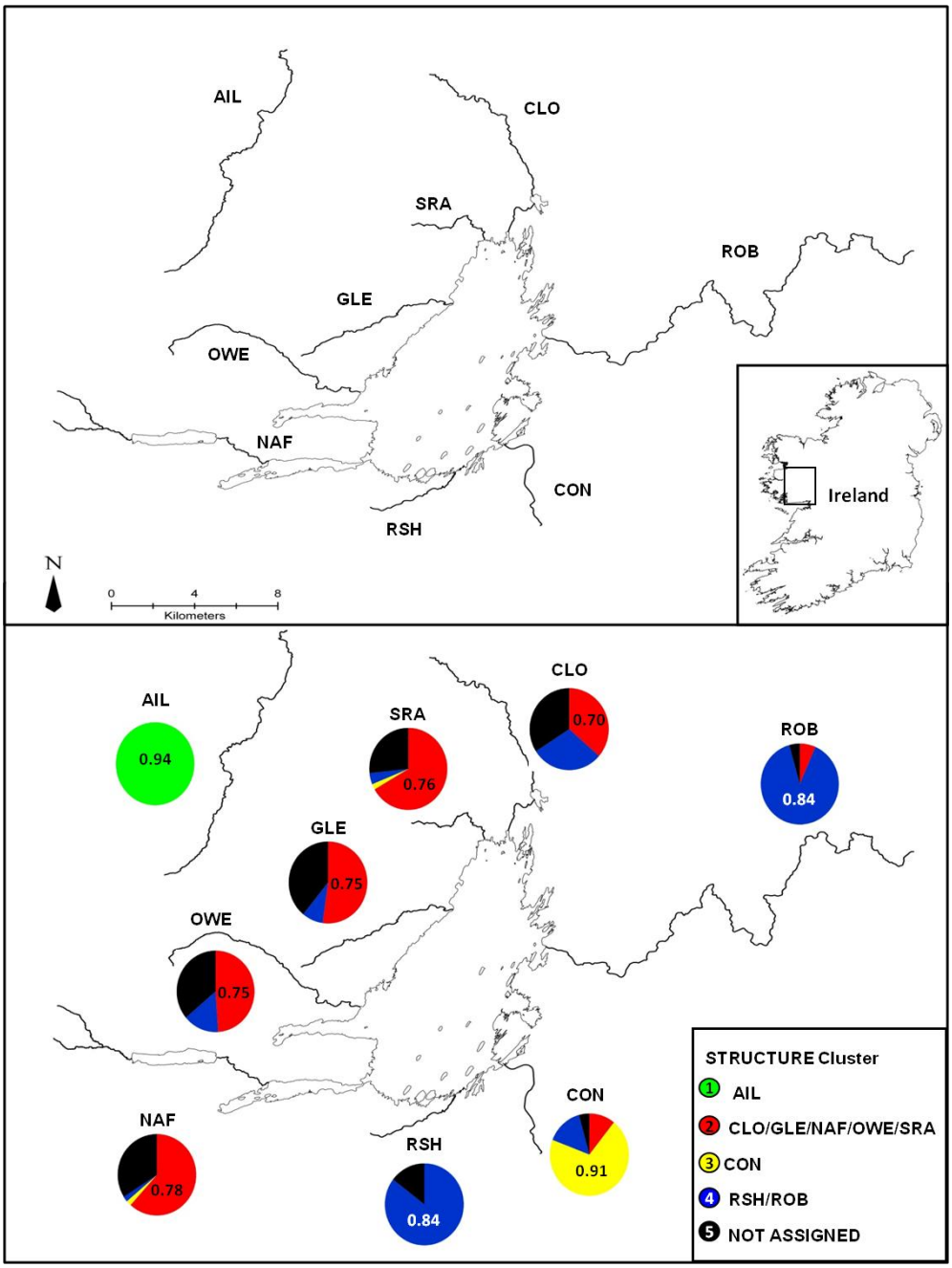

Figure 2.1

a) Inset: location of the Mask catchment in Western Ireland. Main figure: the streams where brown trout were sampled. AIL, Aille; CLO, Cloon; ROB, Robe; CON, Cong canal; RSH, Ross Hill; NAF, Nafooey; OWE, Owenbrin; GLE, Glensaul; SRA, Srah.

b) Geographical distribution of the main genetic clusters identified by STRUCTURE (see text for details). Cluster 1 (green) includes only individuals from River Aille; Cluster 2 (red) is mainly found in the western rivers of the catchment: Nafooey, Owenbrin, Glensaul, Srah and Cloon; Cluster 3 (yellow) only includes brown trout from the Cong Canal; Cluster 4 (blue) is predominantly present in two eastern rivers, Robe and Ross Hill. Numbers in the pies represent the average statistical estimate of 'purity' *Q* of the predominant cluster: for example the green and yellow cluster exhibit greater genetic 'purity' (*Q* close to 1) than fish belonging to the red cluster ($0.8>Q>0.7$). Black colour 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 nine 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 and Petit 1996) and overall and pairwise F_{ST} values using the θ-estimator from Weir and 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 2.2 (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 a membership coefficient (Q) for each inferred cluster that 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. In effect, no individual fish was assigned for Q-values below 0.6. Only individuals with complete data for at least eight loci (N=426) 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. Effective population sizes, that is the likely number of individuals that transmitted their genes to the samples investigated, were estimated using the method based on gametic disequilibrium as implemented in the software LDNE 3.1 (Waples and Do 2008). 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 nine populations sampled. The microsatellites used in this study were highly polymorphic, with 4 (Str60 and STR73) to 32 (OmyFgt1TUF) alleles per locus and an expected heterozygosity across populations ranging from 0.48 to 0.63 (Table 2.1). Levels of genetic diversity (A_R and H_E) were equally distributed across the populations examined, except for AIL, which displayed lower diversity (Table 2.1). CON was the only sample that showed a significantly positive mean F_{IS} value

(Table 2.1). Overall F_{ST} value was high and significant (0.061), indicating strong genetic structuring among the study populations (Table 2.2). The genetic variance was not homogenous among population samples: in fact, AIL and CON showed the highest pairwise values (F_{ST} ranged from 0.221 to 0.080 and from 0.221 to 0.072 respectively) against all other populations, among which instead F_{ST} ranged from -0.001 to 0.062. When the AIL sample was removed, the overall genetic differentiation among populations decreased (F_{ST}=0.041).

Almost all of the F_{ST} pairwise comparisons were significant, after sequential Bonferroni correction, except for the pairs OWE vs. GLE, OWE vs. SRA and GLE vs. SRA (Table 2.2, Fig. 2.2).

STRUCTURE results suggested K=4 as the most likely number of clusters (Fig. 2.3). Cluster 1 is exclusively dominated by individuals from the Aille River. The majority of individuals in Cluster 2 (94%) come from the western rivers of the catchment (Nafooey, Owenbrin, Glensaul, Srah and Cloon); Cluster 3 is largely represented (94%) by the brown trout in the Cong Canal; Cluster 4 mainly corresponds to two eastern rivers, Robe and Ross Hill (Fig. 2.1b). Around 21% of the individuals examined could not be assigned with confidence to any of the five clusters identified. The population that had the highest average membership coefficient to any one cluster (>99%) was AIL (cluster 1) followed by CON (91%) (cluster 3) (Fig. 2.1b). Trout from the western rivers proved to be less genetically 'pure', with a greater proportion of unassigned individuals (between 26 and 37%) and membership *Q*-values below 0.8 (Fig. 2.1b).

Estimates of effective population size, N_e, indicate that populations from the Aille River and Cong Canal are particularly small (Table 2.1).

Table 2.1 Sampling location, sample code and sample size (N) for the studied trout populations. 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;. Bold F_{IS} value refers to significant departure from Hardy-Weinberg equilibrium. Negative estimates of N_e are usually interpreted as infinite estimates (LDNe user's manual).

River	**Sample Code**	**N**	H_E	H_O	N_A	A_R	A_P	F_{IS}	N_e **(95% CI)**
Aille	AIL	48	0.48	0.45	4.17	4.04	0	0.048	85.1 (43.1-373.6)
Cloon	CLO	48	0.60	0.59	7.42	7.05	2	0.003	-733.4 (256.4-∞)
Cong Canal	CON	48	0.56	0.49	7.33	6.93	7	**0.122**	42.5 (32.2-58.9)
Glensaul	GLE	48	0.61	0.61	8.00	7.66	3	0.008	2354.7 (191.9-∞)
Nafooey	NAF	48	0.59	0.57	7.08	6.77	2	0.039	238.4 (103.2-∞)
Owenbrin	OW	48	0.60	0.61	8.25	7.89	6	-0.011	-214.1 (2085.8-∞)
Robe	ROB	46	0.63	0.62	7.00	6.80	3	0.010	287.9 (115.2-∞)
Ross Hill	RSH	48	0.60	0.62	6.50	6.20	2	-0.034	191.2 (90.3 - 9576.1)
Srah	SRA	48	0.58	0.57	7.67	7.38	2	0.020	739.7 (163.0-∞)

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= 0.0014).

	Aille	Cloon	Cong Canal	Glensaul	Nafooey	Owenbrin	Robe	Ross Hill	Srah
Aille		0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003
Cloon	**0.0952***		0.00003	0.00003	0.00003	0.00014	0.00003	0.00003	0.00003
Cong Canal	**0.2214***	**0.0762***		0.00003	0.00003	0.00003	0.00003	0.00003	0.00003
Glensaul	**0.1016***	**0.0136***	**0.0723***		0.00003	0.20106	0.00003	0.00003	0.00850
Nafooey	**0.0796***	**0.0204***	**0.0849***	**0.0140***		0.00003	0.00003	0.00003	0.00003
Owenbrin	**0.0863***	**0.0103***	**0.0818***	-0.0013	**0.0082***		0.00003	0.00003	0.32389
Robe	**0.1632***	**0.0343***	**0.0723***	**0.0391***	**0.0474***	**0.0348***		0.00003	0.00003
Ross Hill	**0.1834***	**0.0403***	**0.0740***	**0.0387***	**0.0616***	**0.0430***	**0.0212***		0.00003
Srah	**0.0846***	**0.0223***	**0.0959***	0.0073*	**0.0107***	0.0012	**0.0470***	**0.0488***	

***** Significance at 0.05 level

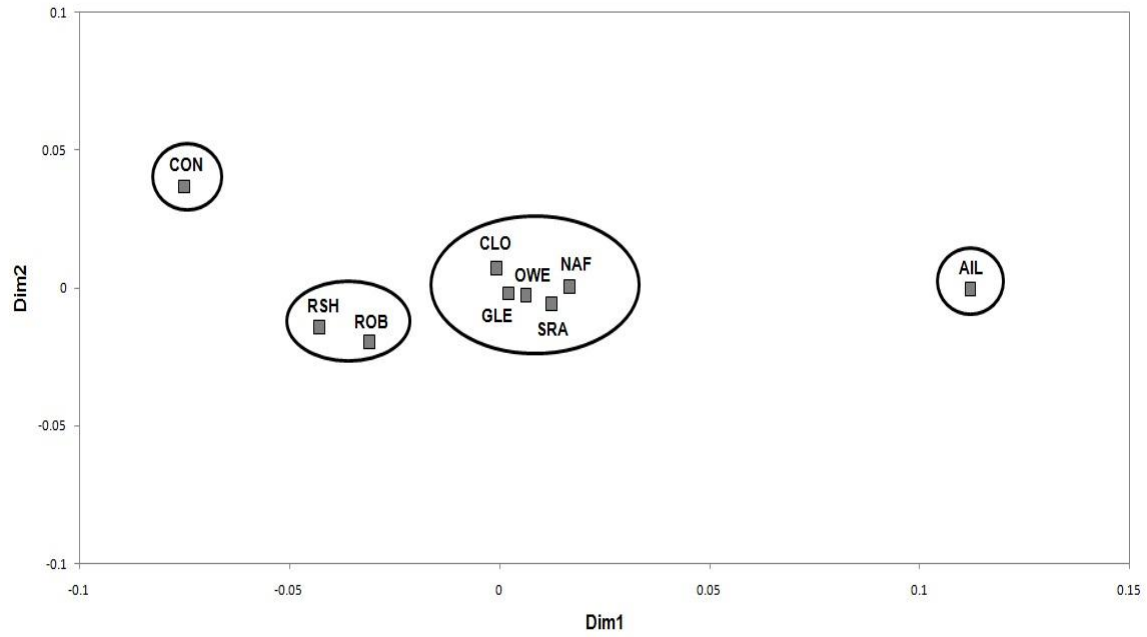

Figure 2.2 Multidimensional scaling plot based on the matrix of F_{ST} pairwise comparisons. The ellipses represent the groups identified by the AMOVA.

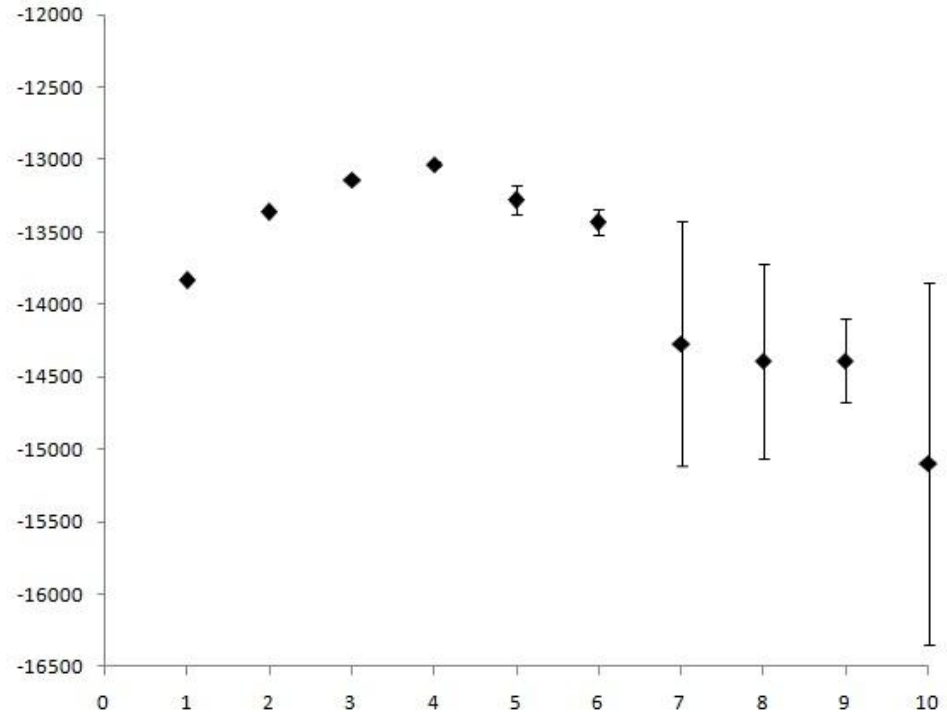

Fig. 2.3 Mean L(K) (± SD) over 10 runs for each K value explored using STRUCTURE showing the lowest variance for K= 4.

3 Lake Stock

3.1 Materials and methods

Mixed fishery data were collected from adult lake trout obtained by either gill netting or angling in 2010 (N= 151). Additional samples of trout exhibiting the "ferox" phenotype were collected in 2008 (N=26), 2009 (N= 29) and 2010 (N=24) by angling (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 2.1.2. 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. All microsatellites used were polymorphic, with 2 (Str60) to 30 (OmyFgt1TUF) alleles per locus. Genetic variability of the Mixed sample was the highest and the lowest values ($A_R < 5$) were invariably recorded for the 'ferox' samples (Table 3.1). F_{ST} pairwise comparisons showed that the mixed stock, MSK10, was virtually undistinguishable from the majority of the fish from the western rivers, whereas the 'ferox' samples were also identical to one another and very closely matching the genetic constitution of the fish from Cong (Fig. 3.2).

STRUCTURE analyses were conducted excluding the isolated AIL population, as this group cannot physically contribute to the lake fishery. The most likely number of cluster was K= 3 (corresponding to clusters 2, 3, 4, identified in the river populations). The proportions of 2010 mixture individuals assigned to the baselines clusters indicated that the highest proportion of trout was assigned to the western rivers (West cluster, 55.63%) and a smaller proportion originated from the East cluster (19.87%). Only 3.97% of the mixed-stock was assigned to Cong Canal. The samples not assigned to any of the baseline clusters were estimated at 20.53 % (Table 3.2, Fig. 3.3). In the 2008 ferox sample 100% of the individuals were assigned to the Cong Canal. In 2009 almost all the individuals (96.3%) were assigned to the Cong Canal with a little contribution from the East cluster (3.70%) and in 2010 the assignment proportions to the same cluster showed a little decrease (91.67%) and the remaining samples were not assigned (8.33%) (Table 3.2, Fig. 3.3).

Table 3.1 Sampling location, sample code and sample size (N) for the samples used in the lake stock 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
Mask 2010	MSK10	151	0.61	0.60	10.08	7.14
Ferox 2008	FER08	26	0.46	0.45	4.67	4.61
Ferox 2009	FER09	29	0.46	0.46	4.92	4.76
Ferox 20010	FER10	24	0.46	0.47	4.75	4.75

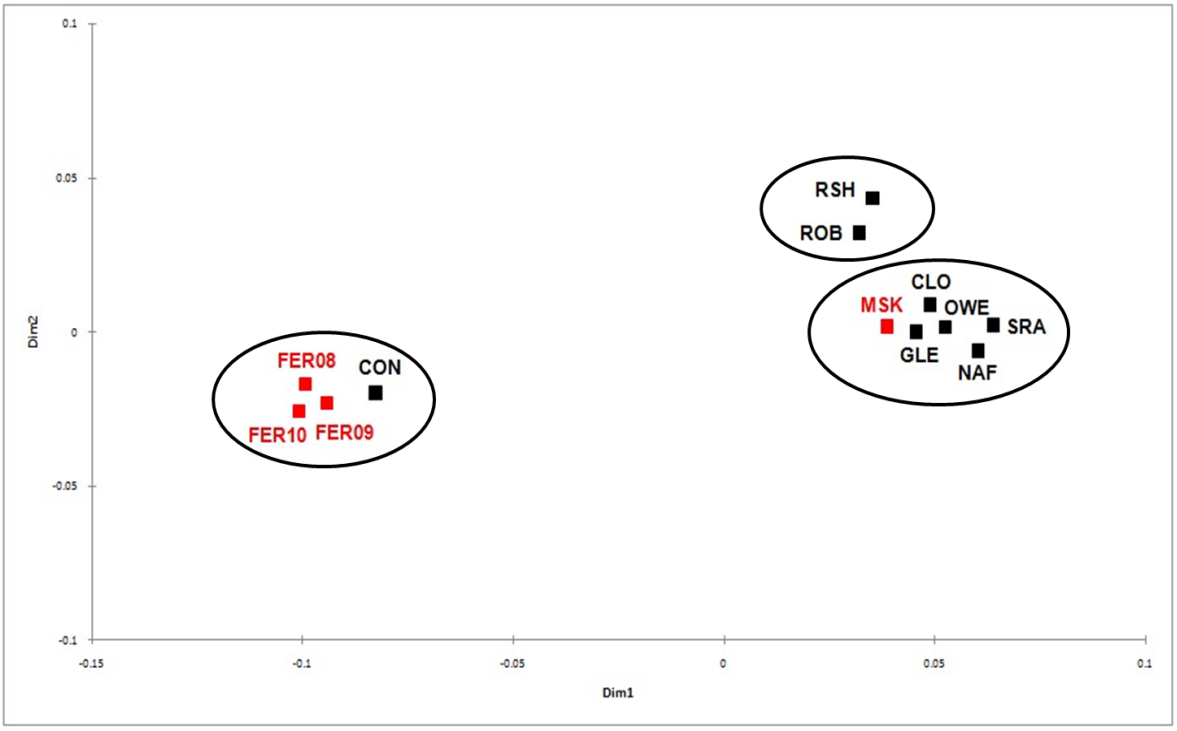

Figure 3.2 Multidimensional scaling plot based on the matrix of F_{ST} pairwise comparisons. The ellipses loosely correspond to the groups identified by STRUCTURE. Mixed and ferox fish sampled from the lake are marked in red.

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 2010 and lake trout identified as ferox collected in 2008, 2009 and 2010. The West cluster is formed by brown trout populations of the rivers Nafooey, Owenbrin, Glensaul, Srah and Cloon, the East cluster includes the brown trout from the Robe and Ross Hill rivers and trout populations in Cong Canal form a single cluster (Fig. 3.1).

	WEST	EAST	CONG	Not Assigned
MSK10	55.63	19.87	3.97	20.53
FER08	0.00	0.00	100.00	0.00
FER09	0.00	3.70	96.30	0.00
FER10	0.00	0.00	91.67	8.33

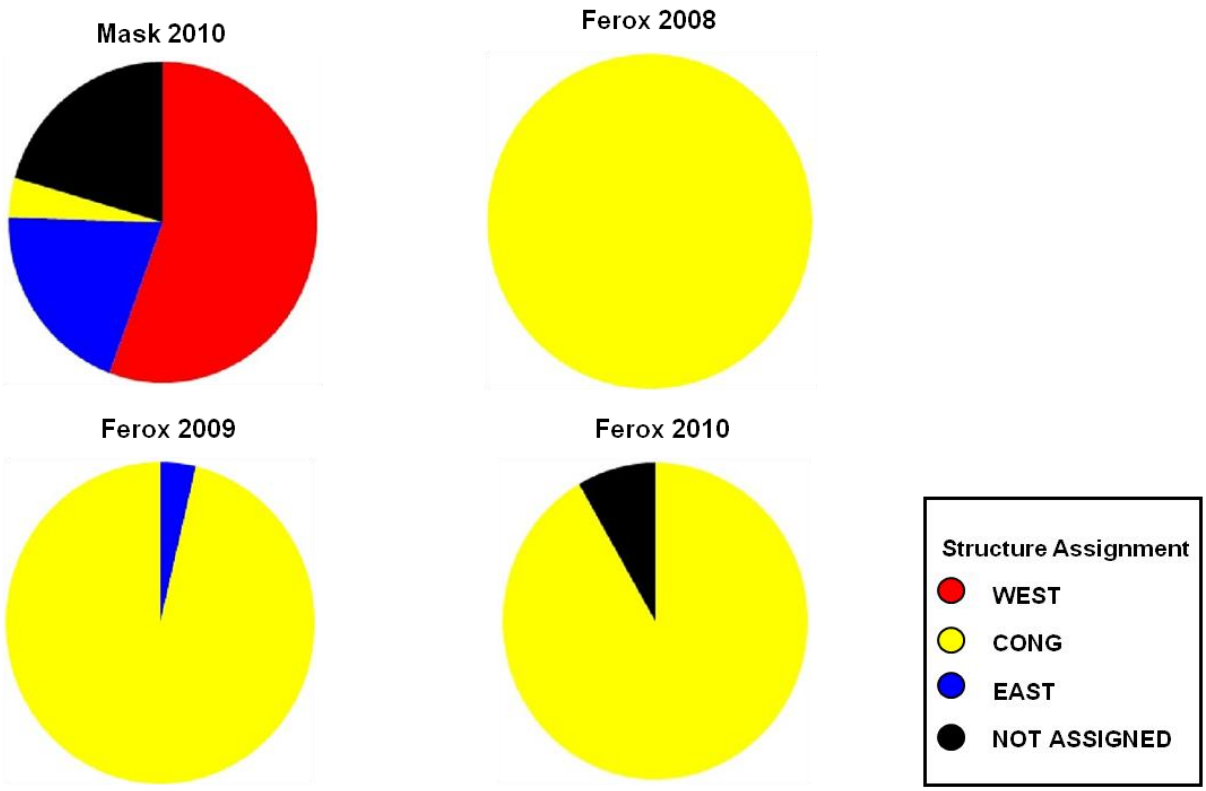

Figure 3.3

Proportions of mixture and ferox-identified individuals assigned to the genetic clusters of baseline populations identified by STRUCTURE. Mixture individuals were collected in 2010, whereas lake trout identified as ferox were obtained in 2008, 2009 and 2010. Colours follow those in Fig. 3.1. Black corresponds to individuals that could not be assigned with confidence to any of the known populations.

4 Conclusions

The present results illustrate with clarity the patterns and the levels of genetic diversity and structuring in brown trout populations from the Mask catchment.

All the main tributaries to the Lough have been sampled, and the results show that the all the genetic diversity found in the Lough is covered by the stream populations. The 'unassigned' component ('black' in Figs. 2.1b and 3.3) likely represents fish that have particularly 'blended' genotypes, deriving from multigenerational straying among rivers. Interestingly, the overall amount of these genetically 'mixed' individuals is around 20% in both the mixed stock and the pooled rivers samples, suggesting almost complete overlap between the genetic diversity sampled in the rivers and that present in the lake.

The Aille river contains a genetically impoverished population likely representing a relict diverged from the rest of the Mask unit in isolation. This population does not exhibit unique genetic feature, but simply the depleted and diverged frequencies of alleles that are expected in a scenario of strong genetic drift. The relatively small estimated effective population size is consistent with a scenario of strong random drift and loss of rare alleles. In the absence of information on striking/notable ecological and phenotypic features of the Aille population, this unit does not appear as a priority for conservation.

Conversely, the very divergent population in Cong ('yellow' cluster in Figs. 2.1) clearly represents an extremely valuable unit that should be carefully protected. Not only is Cong the only river harbouring this genetic cluster, but this also constitutes the gene pool responsible for the 'ferox' phenotype (see Figs. 3.2 and 3.3). The fact that allelic richness is greater in Cong (Table 2.1) than in the ferox lake samples (Table 3.1) is entirely attributable to the existence of non-ferox alleles introduced into Cong by some fish from other clusters (fig. 2.1b). Data not shown also confirm that this genetic cluster is the same as the one identified in the Corrib end of the Cong canal in Massa-Gallucci et al (2010). Given the high economic value of the 'ferox' in both Mask and Corrib, the careful management and conservation of the Cong is of utmost importance, as any environmental damage to this stream may permanently compromise the existence of the ferox in these two catchments.

The western rivers seem to contribute to the largest amount of trout in the lake ('red' cluster); however, the contribution from Robe and Ross Hill should not be disregarded (around 20%). In addition, ROB and RSH also seem to be cohesive units, representing a largely independent cluster, with negligible influx from other rivers. It remains to be discovered what process has led to these rather distant streams to harbour such genetically similar populations.

The genetic features of the western rivers (around 30% 'mixed' individuals and less than 80% 'purity' of the red cluster) indicate that these rivers are more conducive of straying. Some of the 'mixed' fish in these rivers, especially in Glensaul and Nafooey, have some 'ferox' genes, suggesting that occasionally some adult ferox trout may attempt spawning in other rivers, but not establishing a 'ferox spawning cluster', only resulting in some 'hybridization' with other brown trout clusters. These probably are erratic and rare events that are unlikely to allow the establishment of any new ferox spawning ground. The higher propensity to straying in the western side of the catchment and the sheer number of existing streams may be an advantageous feature should any of these streams undergo a populations crash, as they are most likely to be recolonised by the neighbouring rivers (the fact thet SRA, GLE and OWE are genetically undistinguishable support the view of high exchange among these rivers or a very recent common origin of these populations).

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Inland Fisheries Ireland
3044 Lake Drive,
Citywest Business Campus,
Dublin 24,
Ireland. D24 Y265.

www.fisheriesireland.ie
info@fisheriesireland.ie

Tel: +353 1 8842 600