# Population Structure, Genetic Stock Identification and Potential Impact of Farm Stocking

## Lough Sheelin Brown trout

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Iascach Intíre Éireann
Inland Fisheries Ireland

# POPULATION STRUCTURE, GENETIC STOCK IDENTIFICATION AND POTENTIAL IMPACT OF FARM STOCKING - LOUGH SHEELIN BROWN TROUT

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## 1. Introduction

Brown trout (*Salmo trutta*) populations are sensitive to alterations of the physical and natural environment (Elliott 1994). Over the past century, urban growth and associated discharges, farming activities and agricultural run-off, arterial drainage, introduction of alien species, and stocking of farmed fishes, have all contributed to the alteration of the natural aquatic environment and the loss of suitable spawning, nursery and feeding areas for brown trout. These anthropogenic-mediated factors, which have changed both the demography and ecology of local populations, have often led to declines in brown trout productivity in many river catchments in Ireland.

One of the main challenges in the management and conservation of biological resources is to preserve genetic variability (particularly adaptive variation) within and among populations. Adaptive genetic variation is the key feature of populations that enable them to cope with environmental changes and, hence, ensures their long-term sustainability. Since habitat degradation and other anthropogenic activities pose a serious threat to the maintenance of adaptive genetic variation within and among populations, routine genetic monitoring of populations provides important information for the effective development and implementation of both management and conservation plans.

During the late 1950s, an extensive brown trout hatchery stocking programme began in the Lough Sheelin catchment. The impact that such a programme could have had on the wild brown trout population was not known. In 2012, IFI, in partnership with QUB, carried out a genetic study, based on microsatellite DNA profiling, to examine the putative consequences of this management strategy on the population structure of the Lough Sheelin brown trout.

The project was supported by the Lough Sheelin Trout Protection Association (LSTPA), who contributed financially and supplied adult trout scale samples to the project.

The results of this study (2011 and 2012) are reported in here.

## 2. Lough Sheelin Study Area

Lough Sheelin is a medium to large-sized productive lake, located on the border of Counties Cavan, Westmeath, and Meath. It is a relatively shallow alkaline lake with a maximum depth of 15 m. In addition to several minor inflowing rivers (e.g. Crover, Maghera and Carrick streams), the L. Sheelin catchment basically comprises the outflowing Upper Inny River, a main tributary of the River Shannon, and two main inflowing rivers, the Upper Inny and the Mountnugent (Fig. 1). During periods of prolonged dry weather, several of the smaller Sheelin streams (e.g. the Crover, Maghera and Carrick) can experience extremely low water levels with no noticeable flows present at times (*pers comm K. Delanty & B. Montgomery*).

The lake has a surface area of 18.16 km$^2$ and a catchment area of approximately 240 km$^2$. The underlying geology is mainly carboniferous limestone and quartzite (GSI, 2018). Current land use within the catchment is predominately pasture (cattle and pig farming) with some areas of natural vegetation also present (CORINE, 2012). The lake itself is an SPA (Special Protection Area) for several species of birds while the small area south of L. Sheelin is an SAC (Special Area of Conservation) (NPWS, 2018). The lake currently supports populations of brown trout (*Salmo trutta*), pike (*Esox lucius*) and roach (*Rutilus rutilus*), as well as perch (*Perca fluviatilis*), bream (*Abramis brama*), tench (*Tinca tinca*), eels (*Anguilla anguilla*), and hybrids of bream and roach (O'Grady & Delanty, 2000; Connor *et al.*, 2018).

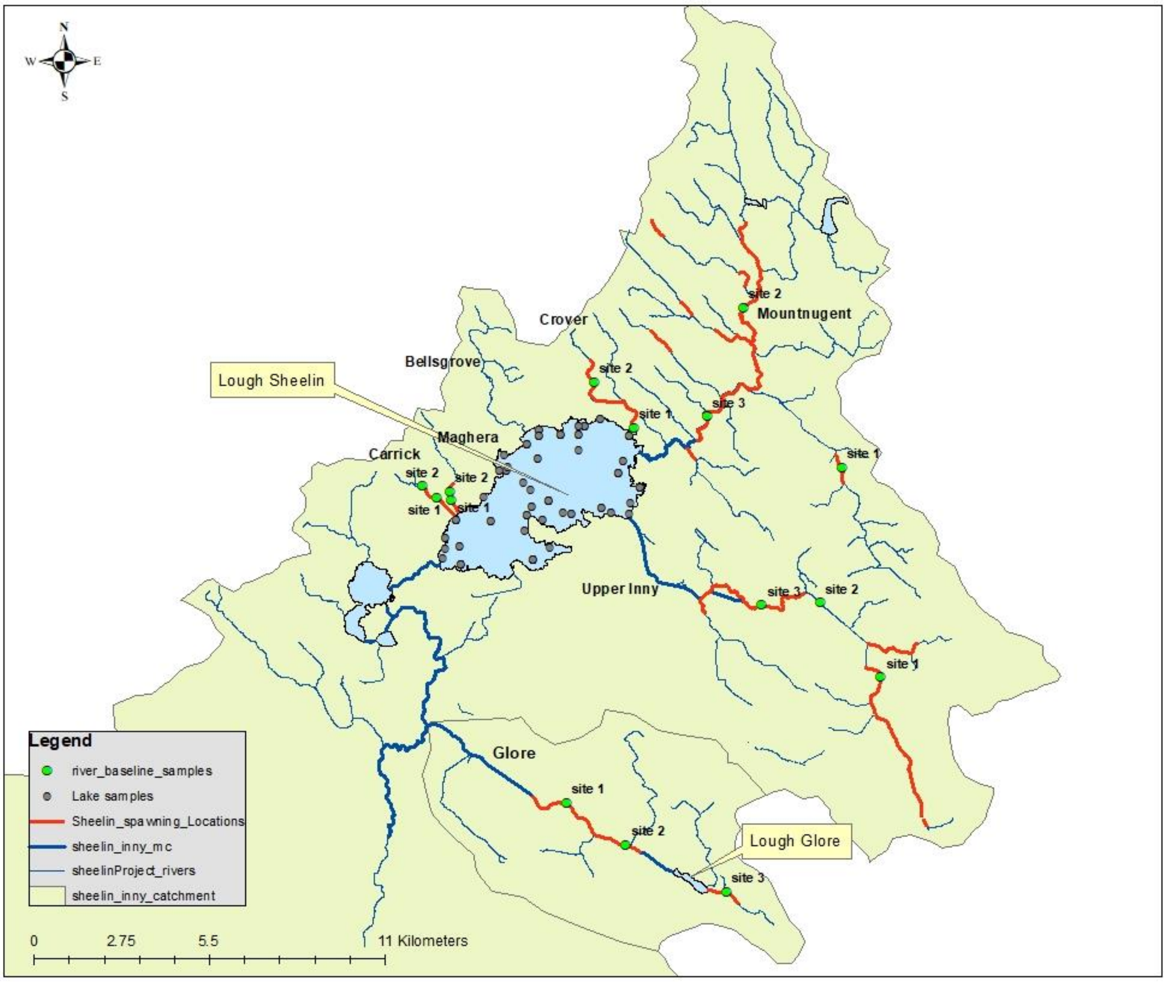


**Figure 1.** Area of study including locations (river & lake) for contemporary sampling, and the main trout spawning areas within the L. Sheelin and L. Glore sub-catchments.

While in the 1960s and early 1970s, L. Sheelin was considered as one of the premier brown trout angling lakes in Ireland, in recent decades, the lake has seen a substantial decline in numbers (Fig. 2). L. Sheelin has experienced many anthropogenically mediated pressures most notably related to poor water quality, and the introduction of roach in the 1970's, possibly as live bait for pike angling (O'Grady & Delanty, 2000). The introduction of roach is considered to have strongly modified fish predator-prey relationships in the system (Shephard *et al*., 2019). Increased agricultural development in the area, during the early 1970s, has led to progressive enrichment (through phosphorus discharge) of the lake that, in turn, both favoured the spread of roach and resulted in a decrease in brown trout numbers (Shepard *et al*., 2019). The first blue-green algal bloom, linked to the progressive enrichment, was observed on Lough Sheelin in March 1971, and since then the lake has shown continuous signs of eutrophication (Champ, 1998; Kerins *et al.,* 2007). In more recent years, L. Sheelin was also impacted by the invasion of zebra mussel (*Dreissena polymorpha*). Zebra mussels were first reported in L. Sheelin in 2001, with the species being fully settled in the lake by 2003 (Millane *et al*., 2012). Paradoxically, considering its invasive status, the zebra mussel, as an extremely effective plankton feeder, is likely to have had a beneficial impact in L. Sheelin water quality (Toner, 2005), which has improved in more recent years. Because of this improvement, the Environmental Protection Agency (EPA) has assigned Lough Sheelin with an ecological status of Good for the purpose of the Water Framework Directive (WFD) reporting 2013 – 2018 (EPA, 2019). Similarly, in 2017, IFI has also assigned the fish status as Good (Connor *et al*., 2018). Nevertheless, given past water quality issues, as well as ongoing river channelisation pressures, L. Sheelin, and much of its river network, has been identified as 'at risk' (EPA webmaps 2019).

Mitigation measures to counteract declining wild brown trout stocks, have mainly focused on an intensive brown trout stocking programme that started in the late 1950's. This programme, which ran for over four decades, involved the annual stocking of unfed fry, yearlings, and adult brown trout of farm origin across the catchment. Between 1957 and 1986, some 4.032 million brown trout (1.4 million fry, 2.5 million 1+ and 0.231 million adults) have been stocked out into the L. Sheelin catchment (IFT Annual reports 1957 to 1980 and IFI unpublished data) (Fig. 3). These figures are likely to be an underestimation, as no reliable stocking activity data exists between 1987 and 2000. The stocking programme ceased completely after 2011. While stocking with hatchery reared fish has been extensively used in Ireland and elsewhere as a mitigation strategy to rebuild depleted populations, there is now increasing scientific evidence arguing both against its usefulness, and its potential negative impact(s) on genetic diversity and fitness of wild populations (Ferguson 2007).

The L. Sheelin river catchment has also been substantially impacted by channelisation. The River Inny arterial drainage scheme (1960-1968) extended as far as the upper reaches of the L. Sheelin catchment including the main inflowing Sheelin tributaries (Fig. 4), though not the Carrick, Maghera and Crover streams. Such drainage schemes have involved a lowering of the natural river bed, over-widening and straightening of the river, removal of instream features such as pools, spawning gravels and natural channel sinuosity. The removal of both instream and bank vegetation and riparian cover were also part of these drainage programmes (O'Grady *et al*., 2017).

As a mitigation measure against habitat changes resulting from channelisation, since the 1980's, and quite extensively during the late 1990's, the network of rivers and streams within the L. Sheelin catchment have been included in several river rehabilitation/enhancement programmes (Tourism Angling Measure (1995-1999) & Environmental River Enhancement Programme (2008-2018), O'Grady & Delanty, 2000). As part of these programmes, measures were implemented, both instream and along the riparian banks, to rehabilitate damaged sections of many of the L. Sheelin tributaries. Approximately 35 kilometres of rivers and streams have benefited from the introduction of spawning gravels, the development of pools and riffle areas, increased channel sinuosity and restricted access of cattle to the rivers (CFB, 1998; O'Grady & O'Leary, 2007; O'Grady *et al*., 2017).

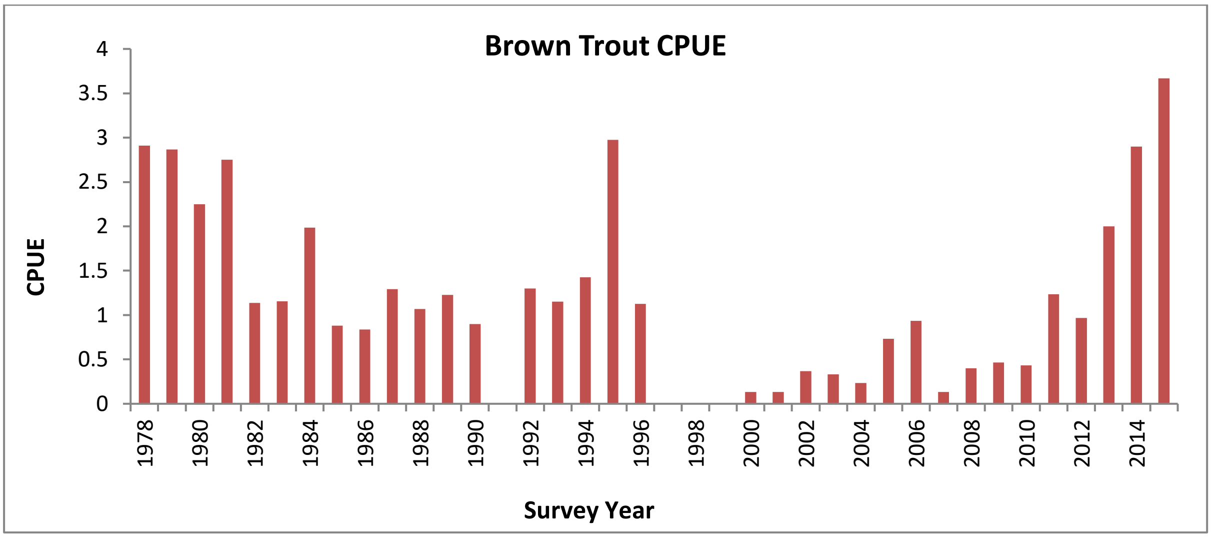


**Figure 2**. Survey netting data - Catch per unit effort (CPUE) for wild brown trout, L. Sheelin 1978 to 2015

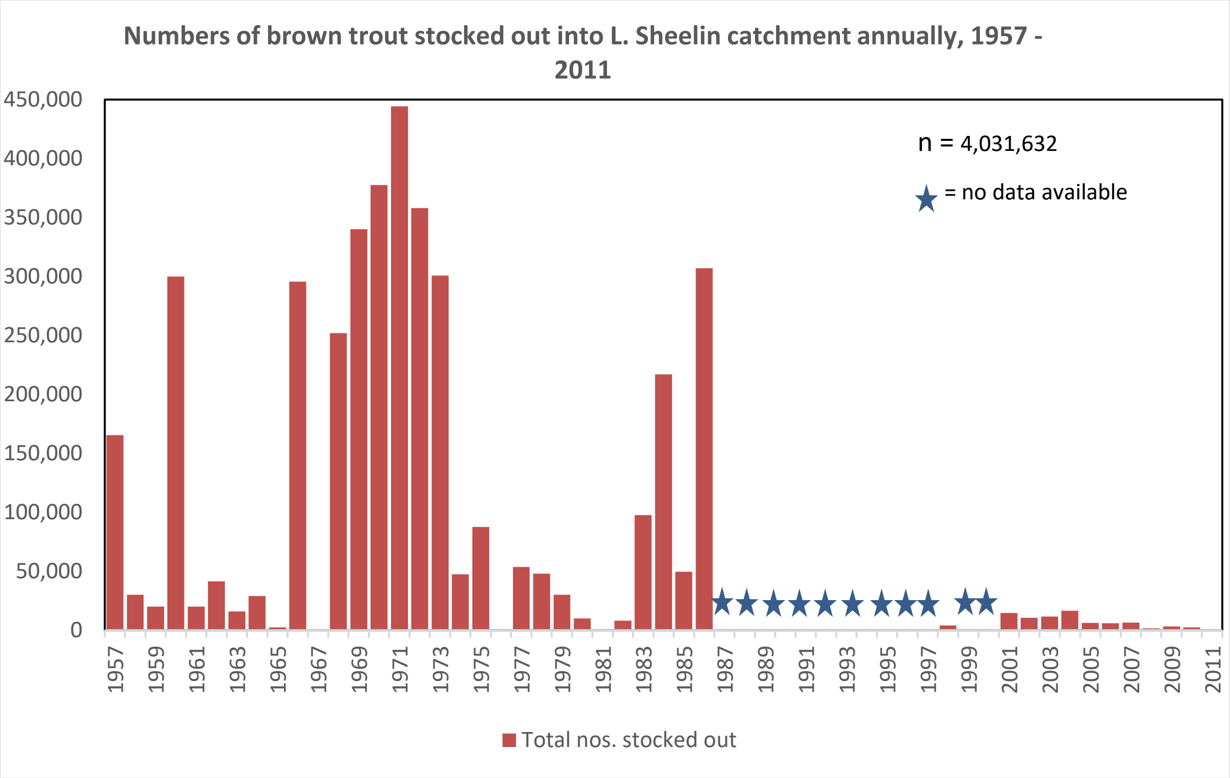


**Figure 3.** Summary of brown trout stocking activities in Lough Sheelin (*stocking of juvenile fish ceased in early 1990's*).

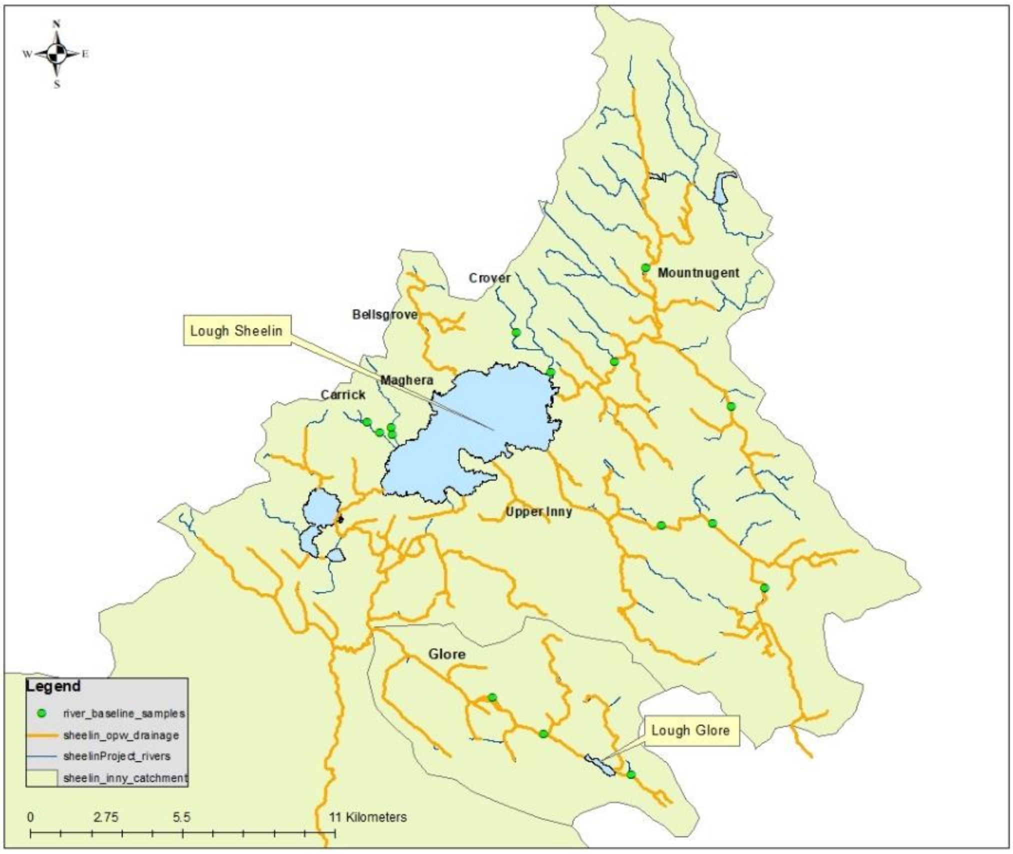


**Figure 4.** OPW drainage network (in orange) within the L. Sheelin & L. Glore sub-catchments.

### Study Objectives

The main objectives of the project were:

- To describe the L. Sheelin contemporary brown trout population structure, and the relative contribution of identified populations to the lake mixed brown trout fishery (Genetic Stock Identification).
- To determine the potential impact(s) of the considerable stocking history in the lake and inflowing tributaries on the wild brown trout populations by comparing the genetic makeup of historical (archived) and contemporary biopsy tissue material collected between 1990 and 2012.
- To determine the putative presence of any upstream migration of brown trout from the Glore River into L. Sheelin.

## 3. Material and Methods

Biological material for genetic analyses consisted of both dry scales preserved in individual envelopes and/or non-destructive biopsy tissue samples stored in 99% molecular grade ethanol. Brown trout (0+, 1+ juveniles and adults) were sampled by electrofishing from five river sub-catchments of L. Sheelin, which were identified as key trout spawning and nursery grounds (Fig. 1). Criteria for sampling location selection involved previously collected data from electrofishing, redd counts, and habitat surveys, carried out over many years as part of the IFI ongoing monitoring river surveys (IFI unpublished data, O'Grady & Delanty, 2000). Biopsy tissue material from the River Glore sub-catchment, downstream of L. Sheelin (see Fig. 1), was also included in the analyses, as there is some suggestion that fish from the Glore system may potentially migrate upstream to L. Sheelin. All baseline river samples were collected by electrofishing nursery sections of the selected tributaries, with a minimum of two sites surveyed within each sub-catchment. Adults (fish larger than 16cm in length) caught in rivers during electrofishing were also non-destructively sampled for analyses. Lake adult brown trout specimens (mixed fishery) were caught using survey gillnets set at different depths throughout Lough Sheelin in 2012 (random survey method - O'Grady & Delanty, 2000; O'Grady, 2012) and from angler caught samples from the 2011 and 2012 angling season (see Fig. 1).

Sampling details are provided in Table 1. A total of 746 specimens were collected between 2011 and 2012. Details regarding fish length, weight, and capture location were recorded in each case. Geographical locations for all sampling sites are shown in Fig. 1. To investigate temporal changes in the genetic composition of populations (i.e. genetic temporal stability), taking into consideration the extensive stocking history of L. Sheelin, historical (archived) biopsy tissue material (N=213) collected by the IFI during the 1990s in the same rivers and geographically close to the 2011-2012 survey sampling locations were also included for analyses as follows: Upper Inny River (N=102, 11 adults and 91 juveniles), Mountnugent River (N=165, 51 adults and 114 juveniles), Crover Stream (N=25 juveniles), Carrick Stream (N= 10 juveniles) and Maghera Stream (N= 26, 24 juveniles and 2 adults). Brown trout archived biopsy tissue material from the Roscrea hatchery were also included in the analyses (N=131) as the baseline for the L. Sheelin stocked fish. Including historical samples, 1078 brown trout were available for analyses in this study.

**Table 1.** Number of fish sampled from each tributary and in total as part of this genetic study (excluding historical-archived material).

| | Site 1 | | Site 2 | | Site 3 | | |
|---|---|---|---|---|---|---|---|
| | Adult | Juvenile | Adult | Juvenile | Adult | Juvenile | Total |
| Carrick Stream | - | 37 | - | 62 | - | - | 99 |
| Maghera Stream | - | 1 | - | 38 | - | - | 39 |
| Crover Stream | - | 37 | - | 13 | - | - | 50 |
| Mountnugent River | - | 20 | - | 30 | 1 | 27 | 78 |
| Upper Inny River | 5 | 24 | 2 | 4 | 5 | 15 | 55 |
| Glore River | 1 | 14 | - | 19 | - | 16 | 50 |
| Lough Sheelin | - | - | - | - | - | - | 244 |
| Roscrea Hatchery | - | - | - | - | - | - | 131 |
| Total | | | | | | | 746 |

Prior to laboratory processing, all sampling information was compiled into an electronic database to facilitate downstream analysis. Genomic DNA was extracted from biopsy tissue material using the Promega Wizard SV 96 genomic DNA purification system and transferred into 96 well microtiter plates for storage at -20$^{o}$C and subsequent genetic analysis. All samples were screened for a marker panel consisting of 18 microsatellite loci (Ssa85, One102a, One102b, CA054565, Ssa416, One103, Cocl-Lav-4, One9ASC, CA048828, CA053293, BG935488, SsaD71, SaSaTAP2A, MHCI, Ssa410UOS, ppStr3, CA060177 and Ssa197) developed and optimised at QUB for brown trout population genetic studies (Keenan *et al*. 2013a). Genetic screening was carried out on a 96 capillary ABI 3730XL DNA analyser following protocols developed and routinely used at QUB. Details on criteria for marker selection and protocols used for genetic screening are described in Keenan *et al*. (2013a). Resulting genotypic data was assembled into an Excel database for subsequent analysis. Further data analyses were only carried out on samples which had consistently amplified for a minimum of 70% of the makers.

## 4. Data Analysis and Results

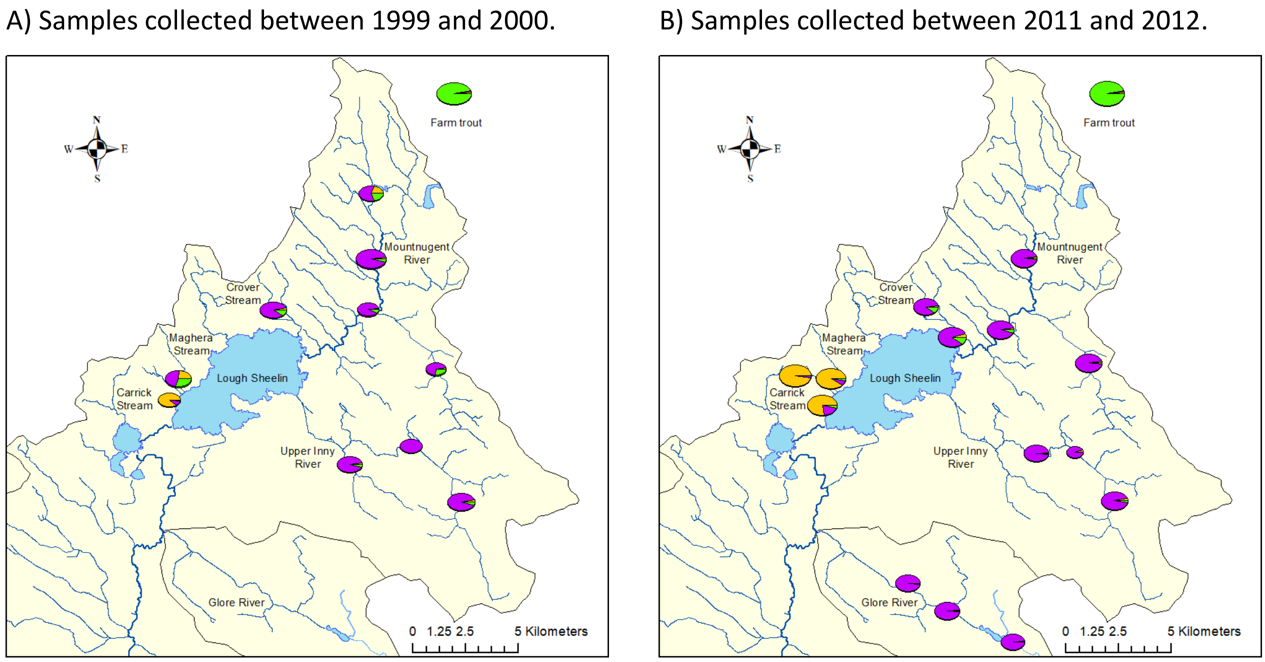
### Data Analysis

COLONY v2.0.5.0 (Jones and Wang 2010) was used to identify potential full-sib individuals within electrofishing samples as a high incidence of full-sibs within samples can lead to a biased assessment of population structuring. There was no evidence for the presence of full-sibs within any of the river/stream samples.

STRUCTURE v2.4 (Pritchard *et al*. 2000) was used to investigate the impact(s) of brown trout stocking in L. Sheelin and examine the patterns of contemporary population structuring. This programme clusters individuals based on their genetic similarities. To ensure an unbiased sample data set for the examination of L. Sheelin brown trout population structuring (i.e. definition of the baseline river populations), adult fish (any individual over the length of 16 cm) were excluded from the STRUCTURE analysis as these may represent transitory (migratory) adult fish. These adults were later used as part of the lake adult mixed stock to assist in the validation of individual population assignment. The rationale was that a large proportion of these adult fish should assign to populations linked to the rivers where they were originally captured.

STRUCTURE was deployed using a hierarchical approach with the objective of identifying major genetic groups within the data (i.e. potentially related by common ancestry) and the subsequent refinement of these down to populations (i.e. genetic baselines). STRUCTURE was initially run with all samples (contemporary, historical and hatchery/farm) in a single analysis to provide an assessment of potential differences in the genetic composition of samples over time resulting from the extensive stocking history. The hatchery sample (Roscrea) was used as the reference baseline for the identification of stocked fish among L. Sheelin samples.

This first stage of the analysis indicates the presence of three distinct genetic clusters explaining the data (Fig. 5). At the first level of population structuring, where equivalent (historical and contemporary) samples are available, the largest cluster occupies the eastern side of the catchment and is represented by samples from the Crover Stream, Mountnugent River, and the Upper Inny River (purple colour in Fig. 5A and 5B). The Glore River samples also group within this cluster (Fig. 5B). The hatchery trout (farm trout) forms the second, independent, very distinct group (light green), while the samples from the smaller streams (Carrick and Maghera) make up the third cluster (predominantly orange) with a clear western distribution. While the samples comprising the eastern group (purple) are remarkably temporally stable (Fig 5A and 5B), some temporal variation is noted in the genetic make-up of the western group comprising samples from the smaller streams (Carrick and Maghera). Despite the extensive stocking history, however, there is no major evidence suggesting substantial introgression of hatchery derived genes into wild groups (i.e. mixing between the genetic make-up of wild versus hatchery fish). Nonetheless, a higher incidence of hatchery derived genes was observed among historical samples collected from several of the rivers of the L. Sheelin catchment between 1999 and 2000. Among the historical samples, the Carrick Stream is characterised by a particularly high proportion of hatchery derived genes (38% contribution). There is, however, little evidence of long-lasting impact(s); thus, samples collected in 2012 contain a much-reduced fraction of hatchery derived genes (2%). A similar trend (i.e. reduction in the presence of hatchery derived genes) was also observed among samples from the other small stream (Maghera), where the genetic contribution has dropped from 28% in the 1990s to 2.5% in 2012. For the larger rivers of the L. Sheelin catchment (Mountnugent and Upper Inny), the average proportion of hatchery derived genes decreased from ~7% in the 1990s to ~1.5% in 2012. It is also important to emphasise that rather than genetic introgression, the presence of “hatchery derived” genes in rivers are mostly explained by individuals, which are clearly of hatchery origin (see below). The overall reduction of the frequency of hatchery derived genes over time in the rivers coincides with the cessation of stocking of fry and juvenile fish in the 1990s.


A) Samples collected between 1999 and 2000.

B) Samples collected between 2011 and 2012.

**Figure 5.** STRUCTURE plots – First level population structuring of the Lough Sheelin baseline river samples involving both historical and contemporary samples. Different colours represent distinct genetic lineages/groups. To facilitate comparison of genetic differences between A) historical and B) contemporary samples, results are displayed in distinct maps. The hatchery baseline (farm trout) is repeated in both plots.

The historical/archived samples were removed from subsequent hierarchical STRUCTURE analyses where the main objective was to investigate contemporary patterns of population structuring and to identify the main population(s) contributing to the lake adult trout stock. The second level of STRUCTURE analysis independently carried out for the groups identified during level 1 (Fig. 5B), provided evidence for further substructuring within samples from both eastern and western groups characterised by a clear geographical pattern. Thus, the eastern (purple) cluster from level one was further partitioned into three sub-groups (Fig. 6).

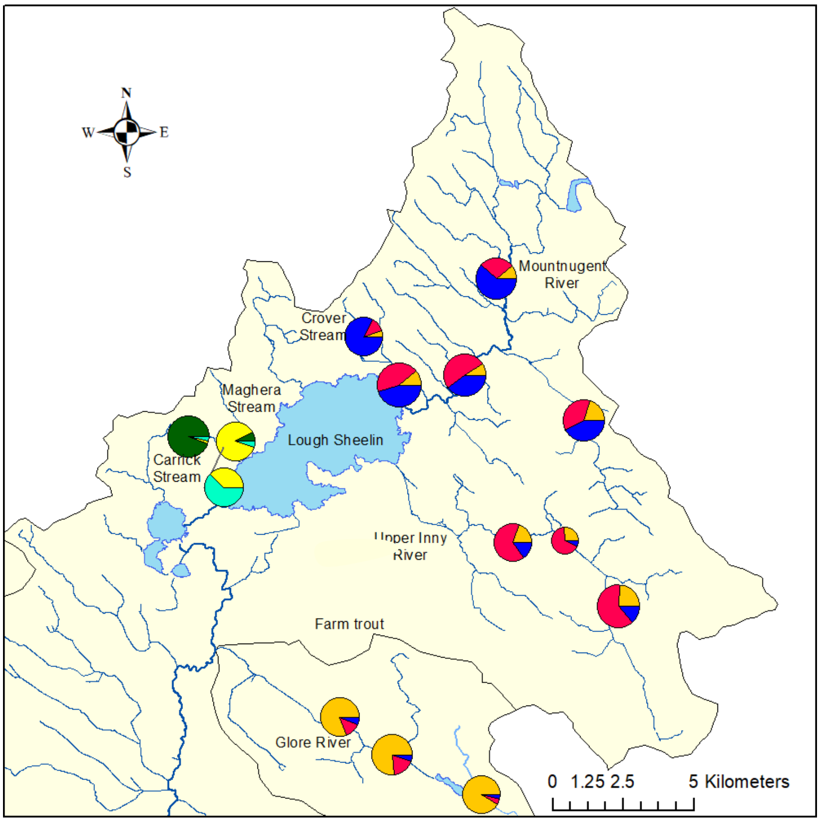


**Figure 6. STRUCTURE plot** – Second level population structuring – of Lough Sheelin baseline river samples involving contemporary samples only. Different colours represent distinctly genetic lineages/groups and the proportion of the colours in the pie chart represent the genetic composition of samples in each case. STRUCTURE analyses were independently run on the ‘purple and ‘orange; groups identified in level 1 (Fig. 5B).

The first comprises samples from the larger Mountnugent River catchment and the geographically close smaller Crover Stream. The second and the third sub-groups comprise samples from the Upper Inny and the Glore River, respectively. Interestingly, there is a noticeable isolation by distance pattern in the genetic make-up of these sub-groups with samples from the first two groups (Mountnugent/Crover Stream and Upper Inny) sharing a greater degree of genetic similarity in comparison to samples from the Glore River. At this level, there is also some evidence of genetic differentiation between samples from the upper and lower reaches of the Crover Stream with the latter sample being noticeably similar to samples from the larger Mountnugent River. The west (orange) cluster from level one is also further partitioned into three distinct groups. The sample from the Maghera Stream makes up the first group while the two samples from the Carrick Stream comprise the second and the third groups respectively (Fig. 6). Additional hierarchical STRUCTURE runs (taking into consideration the groups identified in level 2), did not reveal any major evidence for further population substructuring. Thus, the seven groups identified in level 2 STRUCTURE analysis (excluding the Glore River and Roscrea) represent the current genetic baseline populations for the Lough Sheelin catchment which are basically defined by their respective rivers of origin. Summary statistics (e.g. observed and expected heterozygosity, samples size, allele richness) for these populations are presented in Table 2.

**Table 2.** Summary statistics of inferred baseline populations identified in this study. The sample representing farm trout (Roscrea) is included for comparison. N = no. of samples that have consistently amplified for over 70% used for data analyses; Ar = average allelic richness; Private All = private (i.e. population specific) allele diversity; Ho and He = observed and expected heterozygosities.

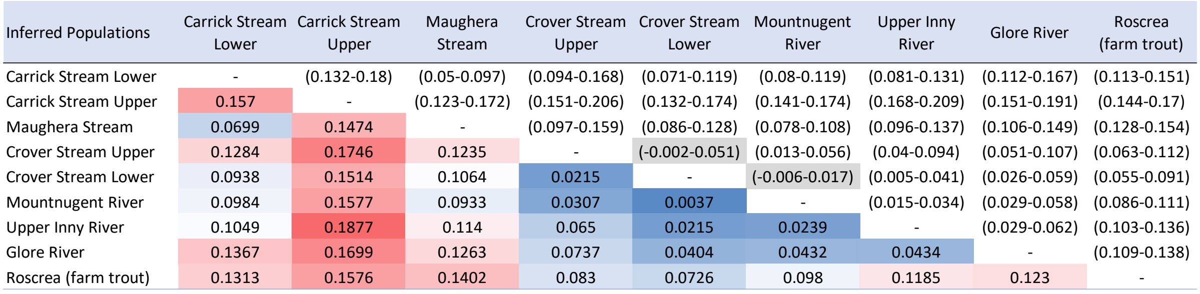
| Inferred Populations | N | Ar | Private All | Ho | He |
|---|---|---|---|---|---|
| Carrick Stream Lower | 37 | 4.41 | 0.06 | 0.626 | 0.56 |
| Carrick Stream Upper | 62 | 3.18 | 0 | 0.667 | 0.504 |
| Maghera Stream | 36 | 3.92 | 0 | 0.64 | 0.554 |
| Crover Stream Upper | 13 | 5.33 | 0.03 | 0.668 | 0.625 |
| Crover Stream Lower | 28 | 7.55 | 0.32 | 0.658 | 0.668 |
| Mountnugent River | 68 | 7.16 | 0.23 | 0.648 | 0.66 |
| Upper Inny River | 45 | 6.56 | 0.2 | 0.613 | 0.627 |
| Glore River | 43 | 5.84 | 0.13 | 0.59 | 0.597 |
| Roscrea (farm trout) | 81 | 7.46 | 0.74 | 0.633 | 0.671 |

The lowest average allelic richness estimates (Ar ~3 to ~4) were associated with the inferred populations linked to the smallest streams (Carrick and Maghera), while the largest values (Ar > 6) were linked to those from the largest rivers. The inferred population group representing Mountnugent River and Crover Stream Lower was characterized by the highest level of allelic richness (excluding the Roscrea farm samples). This is indicative of a larger population size within the larger L. Sheelin sub-catchment. Private (i.e. population specific) alleles were significantly more common in all inferred populations associated with the largest rivers (average Private All > 0.13) while these were either present at low frequency or absent in the smaller streams. Again, this provides evidence for larger population sizes on the larger river catchments. The 'Roscrea' sample has a significantly larger number of private alleles (average Private All = 0.74) in comparison to all other samples. This is not surprising given the "artificial" nature of this particular sample.

The overall $F_{ST}$ was 0.112 (95% CI 0.105 - 0.119) indicating the existence of clear genetic divergence among inferred populations. Significant population pairwise $F_{ST}$ estimates (Table 3) ranged from 0.021 (95% CI 0.005-0.041) between Upper Inny and Crover Stream Lower to 0.187 (95% CI 0.168 – 0.209) between Upper Inny and Carrick Stream Upper. Except for comparisons involving the Crover Stream Lower, no significant genetic differences were noted between samples from Crover Stream and

Mountnugent. It is relevant to note that pair-wise comparisons involving Crover Stream Upper should be treated with caution given the small sample size involved.

**Table 3.** Pairwise Weir and Cockerham $F_{ST}$ estimates (below diagonal) and associated 95% confidence intervals (above diagonal) of genetic divergence between inferred populations. Colour pattern (heatmap) indicates comparative levels of divergence between pair-wise comparisons. "Red" indicates higher levels of between population genetic divergence in comparison to "blue that indicates lower levels". With minor exceptions, all pair-wise population comparisons were found to be significant. The exception involved samples from the Crover Stream lower and Mountnugent River (highlighted in grey) that were found to be non-significant.



| Inferred Populations | Carrick Stream Lower | Carrick Stream Upper | Maughera Stream | Crover Stream Upper | Crover Stream Lower | Mountnugent River | Upper Inny River | Glore River | Roscrea (farm trout) |
|---|---|---|---|---|---|---|---|---|---|
| Carrick Stream Lower | - | (0.132-0.18) | (0.05-0.097) | (0.094-0.168) | (0.071-0.119) | (0.08-0.119) | (0.081-0.131) | (0.112-0.167) | (0.113-0.151) |
| Carrick Stream Upper | 0.157 | - | (0.123-0.172) | (0.151-0.206) | (0.132-0.174) | (0.141-0.174) | (0.168-0.209) | (0.151-0.191) | (0.144-0.17) |
| Maughera Stream | 0.0699 | 0.1474 | - | (0.097-0.159) | (0.086-0.128) | (0.078-0.108) | (0.096-0.137) | (0.106-0.149) | (0.128-0.154) |
| Crover Stream Upper | 0.1284 | 0.1746 | 0.1235 | - | (-0.002-0.051) | (0.013-0.056) | (0.04-0.094) | (0.051-0.107) | (0.063-0.112) |
| Crover Stream Lower | 0.0938 | 0.1514 | 0.1064 | 0.0215 | - | (-0.006-0.017) | (0.005-0.041) | (0.026-0.059) | (0.055-0.091) |
| Mountnugent River | 0.0984 | 0.1577 | 0.0933 | 0.0307 | 0.0037 | - | (0.015-0.034) | (0.029-0.058) | (0.086-0.111) |
| Upper Inny River | 0.1049 | 0.1877 | 0.114 | 0.065 | 0.0215 | 0.0239 | - | (0.029-0.062) | (0.103-0.136) |
| Glore River | 0.1367 | 0.1699 | 0.1263 | 0.0737 | 0.0404 | 0.0432 | 0.0434 | - | (0.109-0.138) |
| Roscrea (farm trout) | 0.1313 | 0.1576 | 0.1402 | 0.083 | 0.0726 | 0.098 | 0.1185 | 0.123 | - |

The quality and usefulness of the identified baseline river populations for individual assignment was examined using the leave-one out test implemented in ONCOR (Kalinowski *et al*., 2007), and the results are summarised in Table 4 and Figure 7. Given the lack of genetic differences involving samples from Mountnugent River and the Crover Stream, these were treated as a single "Mountnugent Reporting Group". The accuracy of the baselines to correctly identify individuals to populations ranged from 84% (Upper Inny) to 100% (Roscrea farm trout) with an average value of 92%. In most of the cases, where misassignments were observed, they invariably involved assignments to a geographically close river baseline. For instance, the highest level (10%) of Mountnugent misassigned individuals were assigned to the Upper Inny (Table 4). Similarly, the highest proportion (16%) of Upper Inny misassignments involved assignments to the Mountnugent baseline population sample. Interestingly, there were some individuals from Carrick Stream Upper misassigning to Carrick Stream Lower but not the other way around. Also, it is worth noting that two individuals (5%) from the Glore River baseline population misassigned to the Upper Inny River population. No misassignments were noted for the farm samples confirming the highly divergent genetic status of fish from farm origin and, thus, providing further validation to assignments to this particular "baseline". Of all baseline river populations, misassignments to the farm sample baseline was only noted for the Carrick Stream Lower (one individual) and Mountnugent River (two individuals).

**Table 4.** Summary results (expressed in %), of correct self-assignment (ONCOR) to river baseline samples (grey diagonal values), including miss-assigned samples (yellow highlighted cells in table).

| Inferred Populations | Carrick Stream Lower | Carrick Stream Upper | Maughera Stream | Mountnugent River/Crover Stream | Upper Inny River | Glore River | Roscrea (farm trout) |
|---|---|---|---|---|---|---|---|
| Carrick Stream Lower | 89% | 0 | 0 | 5% | 0 | 0 | 3% |
| Carrick Stream Upper | 5% | 95% | 0 | 0 | 0 | 0 | 0 |
| Maughera Stream | 4% | 0 | 96% | 0 | 0 | 0 | 0 |
| Mountnugent River/Crover Stream | 0 | 0 | 0 | 87% | 10% | 0 | 2% |
| Upper Inny River | 0 | 0 | 0 | 16% | 84% | 0 | 0 |
| Glore River | 0 | 0 | 0 | 0 | 5% | 95% | 0 |
| Roscrea (farm trout) | 0 | 0 | 0 | 0 | 0 | 0 | 100% |

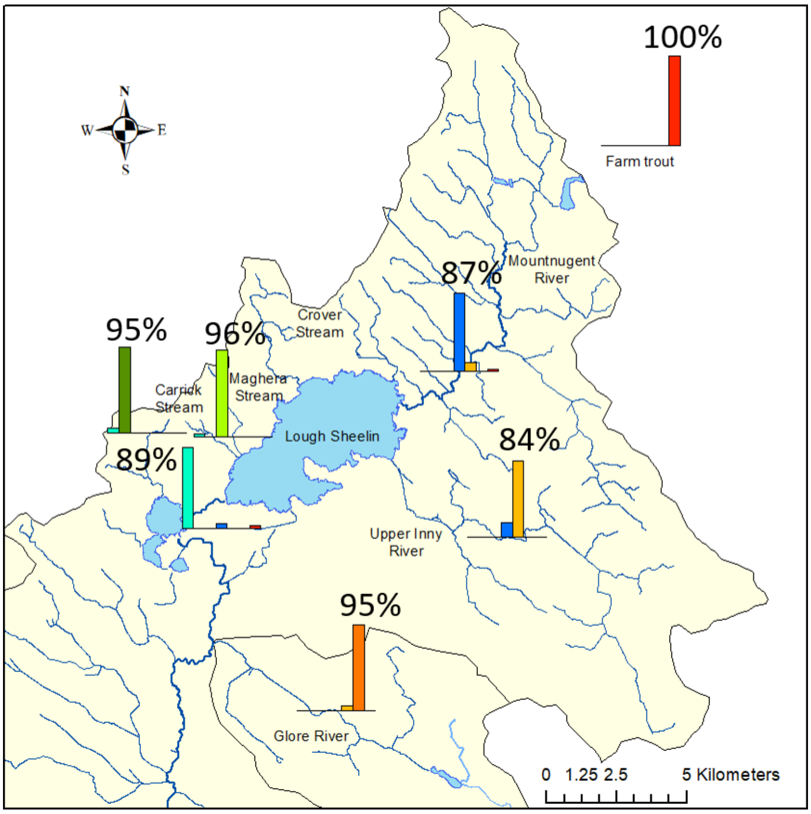


**Figure 7.** Graphical summary of correct self-assignments (ONCOR) expressed in percentage to river baseline samples.

Assignments of adult lake samples caught between 2011 and 2012 (unknown origin) and/or river samples (caught at rivers – mostly deriving from archived/historical material) to the genetic baseline populations, identified in this study, were also carried out with ONCOR. Only adult fish with an assignment probabilistic value (*P*) of 0.7 or higher were considered to be biologically robust assignments. Summary results for adult assignments is provided in Table 5 and Figure 8.

**Table 5.** Summary assignment results for adult lake samples caught between 2011 and 2012, and of all adults caught in rivers (contemporary and historical samples) to the river baseline populations identified in this study (i.e. contribution of baselines to the adult "mixtures"). Both the number and percentages (in parentheses) are provided in each case. 'Unassigned' refers to the number of adults that had poor assignment probability (P<0.7) to any of the identified baselines.

| | *Assign to* | | | | | | |
|---|---|---|---|---|---|---|---|
| Mixture source | Carrick Stream Lower | Mountnugent-Crover 2012 | Upper Inny | Glore River | Roscrea farm trout | Unassigned | Total |
| Lake (2011-2012) | 1 (0.4%) | 135 (60.3%) | 31 (13.8%) | 2 (0.9%) | 41 (18.3%) | 14 (6.3%) | 224 |
| Mountnugent-Crover 2012 | - | 1 (100%) | - | - | - | - | 1 |
| Mountnugent - historical | 1 (3.4%) | 24 (82.8%) | 1 (3.4%) | - | 2 (6.9%) | 1 (3.4%) | 29 |
| Upper Inny 2012 | - | 10 (83.3%) | 2 (16.7%) | - | - | - | 12 |
| Upper Inny - historical | - | 4 (50%) | 3 (37.5%) | - | - | 1 (12.5%) | 8 |
| | | | | | | | **274** |

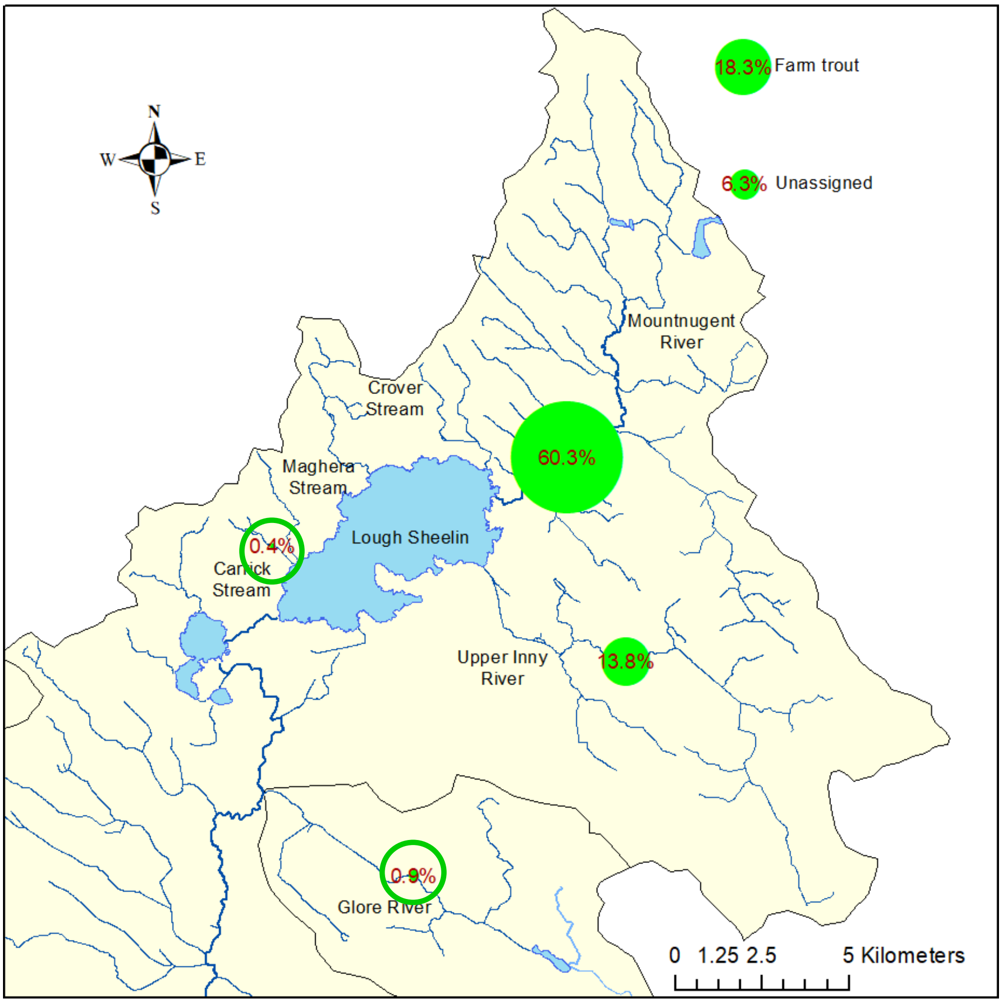


**Figure 8.** Graphical summary assignment results (%) for adult lake trout caught between 2011 and 2012 to the river baseline populations identified in this study.

Results indicate that the Mountnugent-Crover reporting group was the main contributor (60.3%) to the lake (2011-2012) brown trout adult stock, followed by the Upper Inny (13.8%). The contribution of the minor streams was either negligible (0.4% - Carrick Stream Lower) or absent. A small contribution (0.9%) to the L. Sheelin adult stock was also noted from the Glore River population. A noticeable proportion of the lake adult trout (18.3%) assigned to the farm baseline, thus, clearly indicating that these were stocked fish. This observation is not surprising given that stocking into L. Sheelin has only ceased since 2011 and, hence, farm derived adults were still present in the lake during this study. It is also worth noting that a small percentage of the lake fish (6.3%) failed to assign to any of the identified baselines with confidence (i.e. $P < 0.7$). This indicates that there are still one or more unaccounted baseline populations that are also contributing to the lake adult stock. While efforts had been made to ensure that samples from the most likely rivers contributing to the Lough Sheelin lake trout stock were included in the study, there are still a small number of other rivers within the catchment that were not part of the present study (e.g. Bellsgrove River).

## 5. Summary

### Lough Sheelin contemporary population structure (Objective 1)

Since the early 1900s, the Lough Sheelin catchment has experienced several environmental disturbances, which for the most part have had negative impacts on the survival of the wild brown trout populations inhabiting the system (rivers and lakes). Issues have ranged from severe water quality problems, arterial drainage of river network, the introduction of roach and zebra mussel and stocking of farmed brown trout. There is substantial evidence in the scientific literature demonstrating that all these factors can adversely impact population substructuring, genetic diversity and, ultimately long-term sustainability of populations.

The results from this genetic study, however, indicates a remarkable level of natural resilience from the wild brown trout community of the system notwithstanding the many known environmental pressures. Thus, the brown trout inhabiting Lough Sheelin and its river catchment are partitioned into seven distinct populations, which have been shown to be genetically stable (i.e. resilient) over time (Fig. 6). As expected, genetic evidence (i.e. higher levels of genetic diversity) suggests that populations from the larger rivers (e.g. Mountnugent, Upper Inny) are larger in comparison to the smaller streams (e.g. Carrick and Maghera). As a consequence of their larger sizes, they are also more genetically stable over time. The smaller populations (e.g. Carrick and Maghera) are more susceptible to stochastic events (e.g. periodic variation in water levels, low oxygen level) that are likely to drive population size oscillations and, hence, to stronger genetic drift. This is reflected by larger variation in the genetic make-up of these smaller population. It is, nevertheless, interesting to note that despite the temporal genetic oscillations observed in the smaller streams, there appears to be some evidence for a stable "local genetic signature" (i.e. represented by the 'orange' colour in Fig. 5A an B).

The ‘gradient trend’ of population structuring observed among samples from the Crover Stream, Mountnugent, Upper Inny and Glore rivers (see Fig. 6) indicates a clear pattern of isolation by distance, demonstrating that gene flow (natural fish straying) is more common between geographically close populations. This is further confirmed by the patterns of individual misassignments identified during the evaluation of the inferred populations for individual assignment-based analysis. Thus, there is a clear geographical trend with misassigned individuals often assigning to a geographically nearby population. These misassignments most likely represent natural straying behaviour, exhibited by a few individuals of any population, which is characteristic not only of brown trout but also other salmonids. Notwithstanding a certain degree of natural straying, significant genetic differences among populations is likely to be maintained by strong homing behaviour, also characteristic of salmonids.

### Relative contribution of identified populations to the lake mixed brown trout fishery (Objective 1)

In this study, the relative contribution of the various rivers and/streams to the lake mixed trout stock correlates positively with the wetted area of each of the sub-catchments (Table 6). Thus, the larger sub-catchments contribute the most to the adult brown trout populations within the lake. While this positive correlation is not unexpected and has also been noted in the L. Corrib and L. Ree brown trout studies (IFI reports in preparation), other studies have shown this not to be the case; L. Mask (Mariana & Massa-Gallucci, 2010) and L. Derg and Moy studies (IFI reports in preparation). Results from these other studies indicate that brown trout from some of the largest sub-catchments do not migrate to the lake but instead spend their whole life cycle within these larger rivers, migrating between spawning and feeding grounds (e.g. upper and lower reaches of large rivers). These observations are compatible with the wide range of life history variation noted in brown trout (see Ferguson *et al*. 2019 for a review). Thus, wetted area cannot be used as a general criterion for determining putative population contributions to lake adult stocks as each case has to be examined independently.

The presence of unassigned adult individuals within this study suggests that not all populations contributing to the adult lake stock were represented in this study. In this context, one notable absence is the Bellsgrove River. While this river was not considered important for trout spawning and nursery (based on redd count data, habitat type and electrofishing surveys), brown trout have been found in the system and, hence, for completeness should be included in any future sampling programmes and genetic studies to allow for a full review of the Sheelin genetic structure and lake genetic stock assessment.

**Table 6.** **L.** Sheelin sub-catchments, wetted areas and GSI % contribution to the lake adult population.

| River sub-catchment | wetted length (m) | wetted area ($m^2$) | as % of catchment | GSI % contribution to lake |
|---|---|---|---|---|
| Mountnugent | 103933.85 | 277912.33 | 60.61 | 51 |
| Crover | 8640.20 | 17699.11 | 3.86 | |
| Upper Inny | 31961.71 | 126701.74 | 27.63 | 28.1 |
| *Bellsgrove* | *9361.31* | *22806.00* | *4.97* | *na* |
| Maghera | 3997.36 | 8221.59 | 1.79 | 0.4 |
| Carrick | 3209.87 | 5220.18 | 1.14 | |

## Impact of extensive stocking history (Objective 2)

The resilience of Lough Sheelin brown trout stock is further supported by the absence of evidence indicating any long-lasting impact(s) resulting from the known extensive hatchery reared brown trout stocking history within the system. That is, there is no evidence suggesting that this past stocking history, spanning over 40 years, has had any major effect on the local wild brown trout populations. As emphatically demonstrated in this study, farm derived brown trout are genetically very distinct from their wild counterparts and, hence, can be easily identified in the wild. Despite the reported extensive stocking history, this study found no evidence for the persistence of farm derived genes in the wild. Thus, the evidence indicates that, when stocked as fry or juveniles, farm derived fish have extremely poor survival in comparison to the wild fry and/or juveniles, and when stocked as adults, they are either not fit to reproduce or their offspring have again poor survival in comparison to that of wild fish.

Farm-reared brown trout is different from wild brown trout in three main ways. Firstly, they are genetically different because of founding effects, and subsequent domestication in the farm environment, involving artificial selection, relaxed natural selection, and genetic drift. Secondly, they are phenotypically distinct in their behaviour, physiology, and morphology. Finally, they also differ in their learning opportunities especially in relation to feeding and anti-predator behaviour. Given all these differences, it is not surprising that stocking with farm-reared trout results in little or no long-lasting impact on wild populations either in terms of increasing fish numbers or in producing genetic changes (Ferguson, 2007).

The evident recovery of the Lough Sheelin brown trout stock is more likely to be linked to several other factors, in particular improved water quality and habitat restoration. Therefore, current fishery management and conservation should focus on improving environmental conditions of the breeding and nursery areas in the catchment.

### To determine the putative presence of any upstream migration of brown trout from the Glore river up into L. Sheelin. (Objective 3)

The results of this study have confirmed the presence of a small number of brown trout (0.9%) derived from the Glore River in the L. Sheelin adult stock. Thus, genetic evidence confirms previous anecdotal records suggesting upstream migration of Glore River brown trout into L. Sheelin.

### Concluding remarks

While this study was based on samples obtained between 2011 and 2012 and historical samples (1990-2000) data analysis was ongoing for a number of years thereafter. Thus, the characterisation of population genetic structuring of L. Sheelin brown trout and the subsequent individual assignment inferences (i.e. % contribution from each of the main Sheelin river/stream brown trout populations to the adult lake stock) primarily relates to those particular years. Notwithstanding this caveat, it is important to emphasise that the genetic comparisons involving both contemporary (2011 and 2012) and historical samples from the 1990s indicates a remarkable degree of genetic stability over time. Thus, in the absence of any major environmental disturbance in the L. Sheelin catchment over the past few years, it is reasonable to assume that the results (patterns of population structuring and the relative contribution of the identified populations to the lake mixed brown trout fishery) are also temporally stable. Routine genetic monitoring in the future will prove useful in guiding the management and conservation efforts of L. Sheelin brown trout. The most significant finding of this study is that despite the well documented high level of stocking within L. Sheelin, brown trout population recovery was based on wild fish assisted by improved water quality and habitat restoration measures.

## References:

CFB (1998). A Report on the Tourism Angling Measure Trout Habitat Restoration Works. Unpublished internal Central Fisheries Board report.

Champ WST. (1998). Phosphorus/Chlorophyll Relationship in relationship in selected Irish Lakes: Ecological Consequences and suggested Criteria for Ecosystem Management. Cited in Wilson, J.G. (Ed.) 1998 Eutrophication in Irish Waters, pp. 91-105. Dublin Royal Irish Academy.

Connor, L., Coyne, J., Corcoran, W., Cierpial, D., Ni Dhonnaibhain L., Delanty, K., McLoone, P., Morrissey, E., Gordon, P., O' Briain, R., Matson, R., Rocks, K., O' Reilly, S., Brett A., Garland D. and Kelly, F.L. (2018) Fish Stock Survey of Lough Sheelin, July 2017. National Research Survey Programme, Inland Fisheries Ireland, 3044 Lake Drive, Citywest Business Campus, Dublin 24. http://wfdfish.ie/wp-content/uploads/2018/11/Sheelin_2017.pdf

CORINE (2012). https://www.epa.ie/pubs/data/corinedata/

Elliott, JM (1994). Oxford Series in Ecology and Evolution: Quantitative ecology and the brown trout. Oxford Series in Ecology and Evolution; Quantitative ecology and the brown trout, xi+286p.

EPA (2019). https://gis.epa.ie/EPAMaps/Water

Ferguson, A. (2007) Genetic impacts of stocking on indigenous brown trout populations (Environment Agency Science Report No. SC040071/SR). ISBN 978-1-84432-798-0. Retrieved from https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/291703/scho0707bmzi-e-e.pdf

Ferguson, A., Reed, T. E., Cross, T. F., McGinnity, P. & Prodohl, P. A. (2019). Anadromy, potamodromy and residency in brown trout Salmo trutta: the role of genes and the environment. *Journal of Fish Biology* 95: 692-718. https://onlinelibrary.wiley.com/doi/full/10.1111/jfb.14005.

GSI (2018). https://www.gsi.ie/en-ie/data-and-maps/Pages/default.aspx

IFI (2013). Environmental River Enhancement Programme Annual Report, 2013. Inland Fisheries Ireland, Dublin, Ireland.

IFT (1957 – 1980). Inland Fisheries Trust, unpublished Annual Reports 1957 to 1980.

Jones, O. R., & Wang, J. (2010). COLONY: A program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*, 10, 551–555.

Kalinowski, S. T., Manlove, K. R. & Taper, M. L. (2007). ONCOR: software for genetic stock identification. Montana State University, Bozeman, Montana, USA.

Keenan, K., Bradley, CR., Magee, JJ., Hynes, RA., Kennedy, RJ., Crozier, WW., Poole, R., Cross, TF., McGinnity, P**.,** Prodöhl, P. (2013a). Beaufort trout MicroPlex: a high throughput multiplex platform comprising 38 informative microsatellite loci for use in resident and anadromous (sea trout) brown trout *Salmo trutta* genetics studies. *Journal of Fish Biology* 82:1789-804.

Keenan, K., McGinnity, P., Cross, T., Crozier, WW. & Prodöhl, P. A. (2013b) diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution* 4: 782-788.

Kerins, C., Monaghan, K and Champ, WST. (2007). Lough Sheelin and its catchment Water Quality Status and Nutrient Loadings 1998 – 2005. Shannon Regional Fisheries Board, Limerick, Ireland. https://www.fisheriesireland.ie/documents/35-lough-sheelin-and-its-catchment-water-quality-status-and-nutrient-loadings-1998-2005-1/file.html

Mariani, S. and Massa-Gallucci, A. (2010). A genetic study of the mixed trout populations of the Lough Mask catchment. Unpublished IFI internal report.

Massa-Gallucci A., Coscia I., O'Grady M., Kelly-Quinn M. and Mariani S. (2010) Patterns of genetic structuring in a brown trout (*Salmo trutta* L.) metapopulation. *Conservation Genetics* 11: 1689-1699.

Massa-Gallucci, A. and Mariani, S. (2011). A genetic study of the mixed trout populations of the Lough Ennell catchment. Unpublished IFI internal report.

Millane, M., Kelly- Quinn, M., Champ, WST. (2008). Impact of the zebra mussel invasion on the ecological integrity of Lough Sheelin, Ireland: distribution, population characteristics and water quality changes in the lake. Aquatic Invasions (2008) vol. 3, issue 3: 271-281

NPWS (2018). https://www.npws.ie/protected-sites/spa/004065

O'Grady, MF. (2012). The Current Status of Fish Stocks in Lough Sheelin. Inland Fisheries Ireland, Dublin, Ireland. IFI/2012/1-4101

O'Grady, MF. and Delanty, K. (2000). The Current Status of Fish Stocks in Lough Sheelin (March 2000) and A Review of Fluctuations in Fish Populations in this Water over a 22 Year Period and Management Recommendations for its Maintenance as a Trout Fishery in the Long-term. Central Fisheries Board, Dublin, Ireland.

O’Grady, MF., Delanty, K., Coghlan, B., O’Briain, R. and Gilligan, N. (2017). River Enhancement Programmes in Ireland. Inland Fisheries Ireland, 3044 Lake Drive, Citywest Business Campus, Dublin 24, Ireland

O’Grady, MF. and O’Leary, C. (2007). Irish Fisheries Recovery Dataset Provision. Programme of Measures and Standards – Freshwater Morphology. Central Fisheries Board, Dublin, Ireland.

Pritchard, JK., Stephens, M. and Donnelly, P. J. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

Shephard, S., Delanty, K., O'Grady, M. and Kelly, F., 2019. Salmonid Conservation in an Invaded Lake: Changing Outcomes of Predator Removal with Introduction of Non-native Prey. Transactions of the American Fisheries Society, 148(1), pp.219-231.

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