

POPULATION STRUCTURE AND GENETIC STOCK IDENTIFICATION OF

Lough Corrib Brown Trout

IFI/2022/1-4581



Iascach Intíre Éireann
Inland Fisheries Ireland

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OF THE
LOUGH CORRIB BROWN TROUT



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CITATION: Delanty¹, K., Bradley², C., O'Grady¹, M. and Prodöhl², P. A. (2021). Population Structure and Genetic Stock Identification of the Lough Corrib Brown Trout. Inland Fisheries Ireland, 3044 Lake Drive, Citywest Business Campus, Dublin 24, Ireland.

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1. Introduction

Eurasian (brown) trout *Salmo trutta* populations are sensitive to alterations of their physical and natural environments (Elliott, 1994). The Lough Corrib catchment is renowned for its wild brown trout stock, which includes the long-lived, late maturing, piscivorous and highly prized ferox trout (*Salmo ferox*). Over the past century, urban growth and associated discharges, arterial drainage, farming activities and agricultural run-off, introduction of alien species, among other factors, have all contributed to the alteration of the natural lake environment and, the loss and/or fragmentation of suitable spawning and nursery areas for brown trout. The lake has also been associated with an intensive hatchery stocking history, in particular between the mid-1960s and late 1970s. All of these factors, which are known to have an adverse effect on the demography and ecology of local populations, have contributed to fluctuations in brown trout productivity. Consequently, the health status, and long-term viability of trout populations spawning in the rivers comprising Lough Corrib's catchment have been the focus of concern.

To assess the status of contemporary Lough Corrib brown trout populations, in 2006, IFI commissioned a research project to examine the patterns and levels of population structuring and genetic diversity focusing on nine major rivers, which were part of the TAM (Tourism Angling Measure) river enhancement programme. The results of this project have been reported by Massa-Galluci *et al.* (2010). In 2012, the IFI commissioned QUB (Fish Population Genetics Research Group) to carry out a follow-up genetic study on the Lough Corrib brown trout with the aim of confirming the results of the initial survey, and to investigate possible changes in the genetic make-up of populations as a consequence of the changing environment. This new study is based on a new large-scale biological survey of Lough Corrib and its main tributary rivers and streams. A key distinction between the Massa-Galluci *et al.* (2010) and the 2012 survey is that, for the latter, the exact location of each lake adult brown trout is known in detail. Another relevant distinction of the present genetic study is that opportunistic historical archived tissue material (brown trout scales collected from IFI fish surveys in 1974 and between 1994 and 1998) was also available for analyses. This archived material allows for a direct assessment of putative genetic changes among L. Corrib brown trout populations over a twenty years period.

The results of this more comprehensive study are reported here.

2. Lough Corrib Catchment Study Area

Lough Corrib is the second largest lake in Ireland (after Lough Neagh in Northern Ireland) and is situated in Co. Galway in the River Corrib catchment. The main tributary rivers draining into L. Corrib include the Cornamona, Bealanabrack, Owenriff, Drimneen, Cong, Clare, Annacourta, Black and Cross rivers. In addition to its own catchment area, Lough Corrib also encompasses the catchment areas of both Lough Mask and Lough Carra. Lough Carra connects to Lough Mask via the Keel River outflow while Lough Mask drains into L. Corrib via underground channels and aquifers (GSI, 2021), and through the man-made Cong Canal surface connection that was built around the late 1840s / 1850s (Wilkins, 1989).

Prior to the 1850s, there was no direct surface river connecting Lough Mask to Lough Corrib. Water flowed underground through karst limestone from several points on Lough Mask's southern shore and re-emerged via springs in the village of Cong. The emergence of this underground outflow from Lough Mask forms the Cong River, which flows for some 1.25 km before entering Lough Corrib. During the late 1840s the Commissioners of Public Works (now the Office of Public Works - OPW) commenced excavation of a navigation canal between Lough Mask and Lough Corrib. Excavation continued as far down as Cong village but the project ceased in 1854 due to the porous nature of the underlying limestone. Since then, this canal has served as an overground connection between the two lakes during high water conditions. To prevent adult salmon moving upstream through the man-made canal to Lough Mask, a fish fence/screen has been in place since the 1940s (Gargan *et al.*, 2020).

For the purposes of this report, from here on, the L. Corrib catchment will only include the lake (L. Corrib), and its inflowing rivers and streams (including the Cong River and Cong Canal) but not the L. Mask or L. Carra systems (Fig 1.). The exception to this has been the inclusion of ferox trout from L. Mask in the analyses of the present study. Given the direct and indirect connections outlined above, there is interest in examining the genetic relationship between ferox trout from both L. Corrib and L. Mask.

The Lough Corrib catchment drains a vast area of approximately 2242.75 km². The lake itself covers an area of 176 km² and, its largest river sub-catchment, the Clare River (and associated tributaries), an area of approximately 1094 km². The catchment is underlain by a number of differing geologies ranging from limestone (east of L. Corrib) to granite and schist (west of L. Corrib) and to the north, quartzite (GSI 2018). Land usage within the catchment is also noticeably divided based on west and east of L. Corrib. The surrounding lands to the south and east are mostly pastoral farmland, while bog and heath predominate to the west and north.

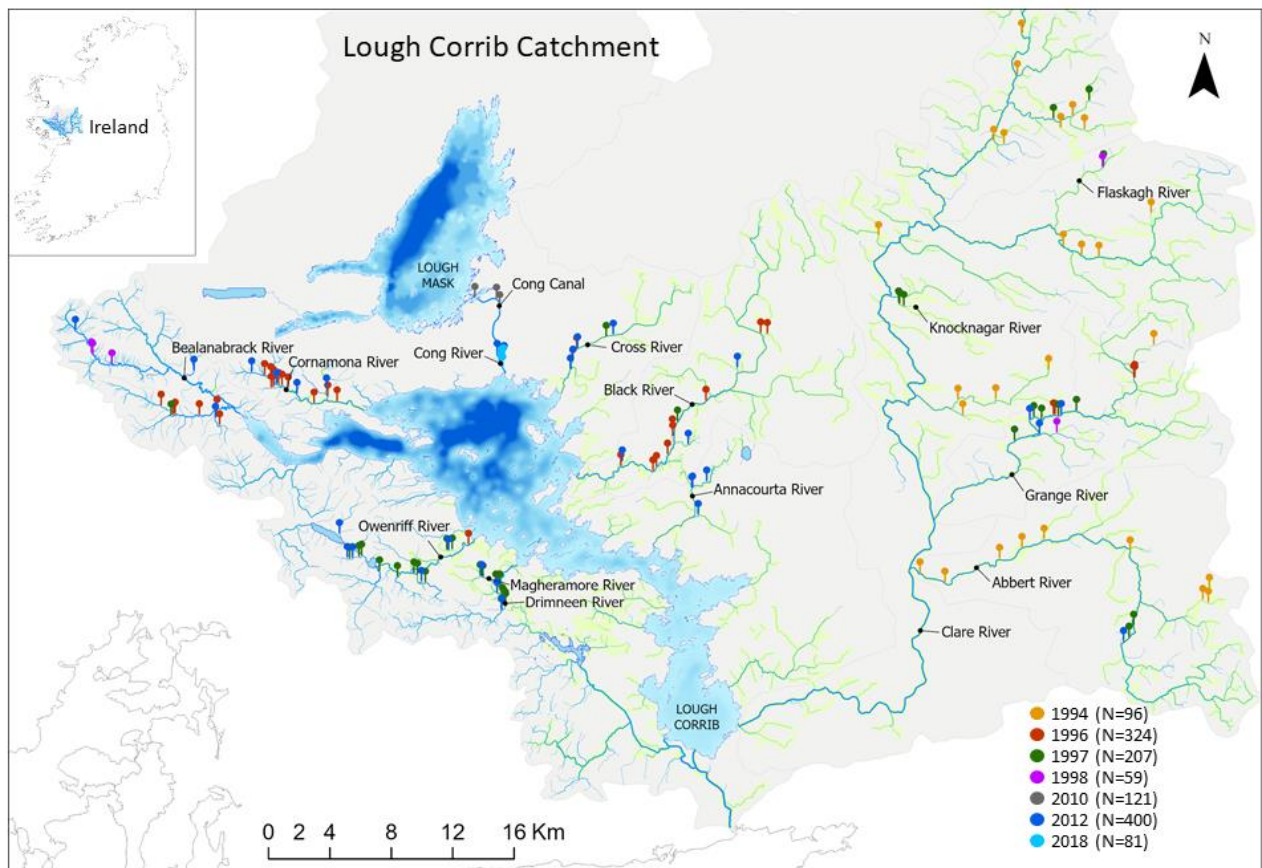


Figure 1. Lough Corrib river catchment (area of study) including river sampling locations (coloured pins). Different coloured pins indicate sampling year. Rivers affected by OPW drainage schemes are highlighted in the map (yellow shade) and also in Appendix IA. Lake depth (L. Corrib and L. Mask) is illustrated by the blue colour gradient (shallow and deep areas in the lakes are represented by lighter and darker blue colours respectively).

Three large scale drainage schemes were completed across the Corrib Catchment by the OPW between 1951 and 1986 as follows: 1- Corrib-Clare scheme (1951 to 1959), 2- Corrib-Headford scheme (1967 to 1973), and 3- Corrib Mask scheme (1979 to 1986) (Appendix A). These drainage schemes impacted rivers and streams across the whole of the eastern side of the catchment (particularly affecting the Clare River sub-catchment), an area where agriculture was more significant (CORINE 2018 (Appendix B). In comparison, only sections of the western and northern end of the catchment were included in the schemes (See Fig. 1 and Appendix A). The drainage schemes typically involved a lowering of the natural riverbed, over-widening and straightening of the river, and removal of instream features, e.g. pools, bank vegetation, spawning gravels, natural channel sinuosity (Gargan *et al.*, 2002; O’Grady *et al.*, 2017).

Prior to these drainage schemes (20th Century), historical drainage works were undertaken by the Commissioner of Public Works, in the 19th Century, on both the Cong Canal (1848-1854) and the Clare River (1840s – to 1850s). The Clare River catchment lies within an extensive area of karstic limestone

and, as such, the Clare River has been described as not being a 'natural river', but more like an 'aqueduct' linking a series of pre-existing lakes, turloughs and reaches of stream. For instance, prior to arterial drainage in the 19th century, the River Abbert sank underground at Ballyglunin, and the River Clare sank underground at Turloughmore (Appendix C). A significant volume of the River Clare flow sinks underground and continues westwards, re-emerging as springs, such as Bunatober and Aughcloggeen, on the eastern side of Lough Corrib (EPA, 2018) (Appendix A). Other reports state that *'Originally, prior to the 1840's drainage scheme the Clare disappeared underground in three streams at Rusheens South, five miles south of Tuam to emerge eight miles further south at Cregmore. There were two main swallow holes at Pollakilleen and Pollnacloya. This extraordinary regime with three inflowing rivers, a turlough lake complex with numerous swallow-holes and underground flow was probably unique in Ireland, if not in Western Europe'* (D'Arcy, 2000 & Hurley, 2017). The opening up of the Clare River system during this period resulted in the original river course being changed to that which we see today (Appendix A). Much of this drainage works involved blasting and excavating a new channel both through the turlough upstream of Turloughmore and continuing downstream re-connecting to the original river course around Cregmore (Hurley, 2017 & Wilkins, 1989). Access for salmon to the upper reaches of the Clare River, prior to those works, was prevented by a narrow cataract located near Turloughmore, which caused the retention of water resulting in the formation of the large turlough upstream of it. This cataract was also blasted during the excavation works, creating a clear and open passage that allowed fish to move freely from L. Corrib up through the entire River Clare system (Wilkins, 1989).

The Lough Corrib catchment has a long history/association of stocking juvenile trout from local hatcheries using wild Corrib broodstock and from IFI's own fish farm at Roscrea (juveniles and adults) into both the lake and many of its inflowing tributaries. The hatchery at Oughterard on the Owenriff River has been in operation since 1852. It started as a salmon hatchery unit and became a trout hatchery at the turn of the 20th century. The Oughterard hatchery is owned by the Lough Corrib Angling Federation and is run on a voluntary basis by local anglers. Every year, at the end of October, nets are laid across the Owenriff River where, over the course of one week, adult broodstock (male and females) are taken to the hatchery to be 'stripped' and fertilised ova developed (Oughterard Hatchery, 2021). This operation is carried out under license from the Dept. of Communication, Climate Action and Environment. The number of adults taken for broodstock has varied over the years ranging from 38 to 360 males, and 109 to 500 females. Since 2015, it has been capped at 200 males and 200 females, which on occasion is not reached. These adult fish are then released back to Lough Corrib shortly after the stripping process has been completed. Between January and February, the ova develop into fry and somewhere between 150,000 to 300,000 unfed trout fry were released

throughout the Corrib system (rivers and lake), on a yearly basis, with all Federation Clubs receiving fish for their 'waters'. Since 2015, however, the release of unfed brown trout fry produced by the Oughterard Hatchery is now restricted to the Owenriff system.

A second hatchery is located on the Cong River within the village of Cong. This unit, which is managed by IFI (and its predecessors Inland Fisheries Trust & Western Regional Fisheries Board), has been in operation since 1962 (IFT, 1957 – 1985). While the Cong hatchery is currently a freshwater salmon smolt rearing facility operating a salmon ranching programme, it was originally built to facilitate the rearing of salmonids, which included brown trout, to the one plus stage, known as yearlings (CFB, 1983). The original hatchery was implemented as a mitigation measure to offset the potential impact of the OPW drainage work programme, ongoing across the Corrib catchment (1950s to 1980s). Thus, the role of the facility was to produce yearlings to be stocked out into rivers drained by the OPW. The trout broodstock consisted of wild brown trout that were primarily sourced locally from the Cong River itself, but also across several other Corrib tributaries, most notably the Owenriff River via the Oughterard hatchery and, on occasion, also from Cornamona, Maumwee and Clare rivers. The resultant 1+ brown trout (yearlings) were then distributed across several Corrib tributaries and the lake (*pers comm. M. Varley & P. Gorman, IFI*). The hatchery ceased producing trout for stocking in the mid-1990s.

The third source of stocked brown trout came from the IFI operated Roscrea Fish Farm (Co. Tipperary). During the 1960s, 1970s and up to the early 1980s, L. Corrib was annually stocked out with farmed reared trout fry (0+), summerlings, yearlings and 2+ yr olds (IFT annual reports 1957 – 1987). Approximately 1.6 million fish (of all life stages) were stocked out into L. Corrib and its main tributaries between 1966 and 1987 (Fig. 2).

Additional mitigation measures focused on habitat improvement. Since the 1980s, and extensively during the late 1990's, the network of rivers and streams within the Lough Corrib catchment have been included in several river rehabilitation and/or enhancement programmes (Appendix D) (Tourism Angling Measure 1995-1999 & Environmental River Enhancement Programme 2008-2018). As part of these programmes, measures were implemented, both instream and along the riparian banks, to rehabilitate damaged sections of many of the L. Corrib tributaries. Approximately 75 kilometres of rivers and streams have seen the introduction of spawning gravels, the development of pools and riffle areas, increased channel sinuosity and restricted access of cattle to the rivers (Duff, 1997; CFB 1998; Gargan *et al.*, 2002; O'Grady *et al.*, 2002; O'Grady & O'Leary, 2007).

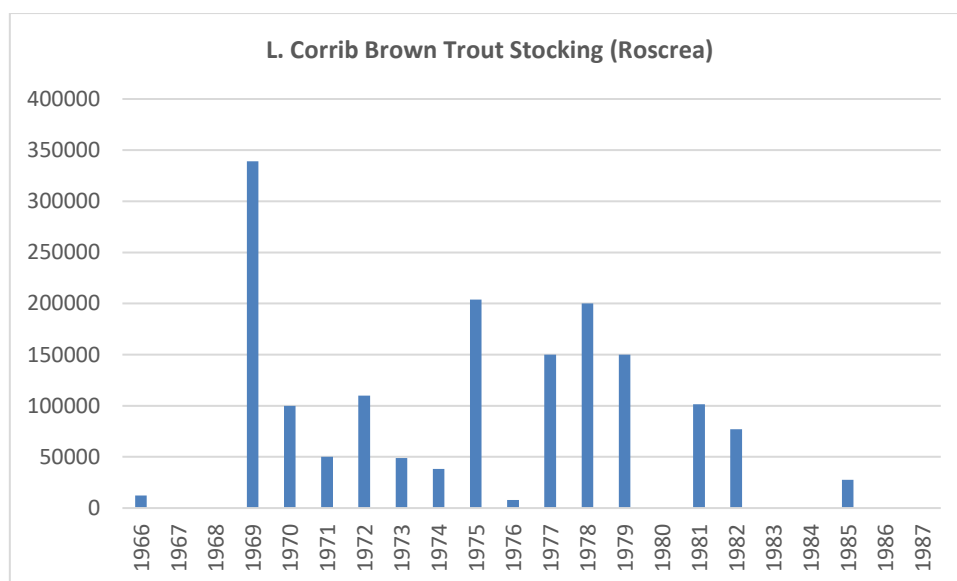


Figure 2. Summary of brown trout stocking (Roscrea derived fish) activities in Lough Corrib between 1966 and 1987.

Listed within, or part of the Lough Corrib Catchment, are several important Special Areas of Conservation (SAC) including the Lough Corrib SAC, the Connemara Bog Complex and the Maumturk Mountains (Appendix E). These SAC's are regarded as areas of significant importance for a range of habitat types, flora and fauna including salmon and lamprey species (NPWS, 2018). Several rivers (and associated tributaries) including, the Clare, Grange, Abbert, Sinking, Dalgan and Black to the east, as well as the Cong, Bealanabrack, Failmore, Cornamona, Drimneen and Owenriff to the west are included within the Corrib SAC due to their importance for Atlantic Salmon (NPWS, 2018). Water quality across the catchment has varied considerably over the past decades. According to the most recent EPA water quality assessment, for the period between 2013 and 2018, much of the catchment is at "Good Status" (EPA, 2018) (Appendix F).

Study Objectives

The aims of this follow up genetic study in Lough Corrib were:

- To confirm and further characterise the brown trout population genetic structure within the Lough Corrib catchment, and to establish a genetic population baseline to:
 - Using Genetic Stock Identification (GSI), identify the main brown trout populations, and associated tributary rivers, contributing to the lake adult mixed brown trout stock (and fishery).

- Use as a reference to monitor changes linked to both future environmental fluctuations (e.g. habitat alterations, climate change) and/or management approaches (e.g. stocking).
- To test for possible association between location of captured adult fish from the lake mixed brown trout stock and their population of origin. That is, to test whether adult fish, in the lake, tend to remain close to the specific tributary river linked to their population of origin.
- To assess possible impact(s) of the extensive stocking history in the lake and inflowing tributaries on the wild brown trout populations.
- To quantify patterns and levels of gene flow between identified populations (i.e. genetic connectivity between populations).
- To identify the main tributary(ies) river(s) contributing to the Lough Corrib ferox trout and to examine whether there is a connection between ferox trout from L. Corrib and L. Mask.

3. Material and Methods

Nine main Lough Corrib tributary rivers (Cornamona, Bealanabrack, Owenriff, Drimneen in the West, Cong in the northeast, and Clare, Annacourta, Black and Cross in the East), and associated sub-tributaries were selected for sampling (Fig. 1). Rationale for river and/or tributary selection, including sampling sites, was based on electrofishing surveys, redd count surveys and habitat surveys, carried out over several years (K. Delanty *pers comm*, Gargan *et al.*, 2002). These rivers were also previously sampled as part of the Massa-Gallucci genetic study (Massa-Gallucci *et al.*, 2010). The entire lake (upper and lower basins) was also sampled following a random survey approach (O'Grady *et al.*, 1996, O'Grady & Delanty, 2013).

Sampling for all biological material used in this study spanned a 34 year period (1974 to 2018), and it was co-ordinated by IFI. Biological material consisted both of dry scales stored in individual envelopes and/or non-destructive biopsy tissue samples stored in collection containers filled with 99% molecular grade ethanol.

River Baseline Samples

Biopsy sample material for the identification of baseline river populations consisted of 926 juvenile brown trout (≤ 16 cm in fork length and 0+ & 1+ in age) scales caught by electrofishing between 1994 and 2018. Brown trout scales, from fish collected between 1994 and 1998, were part of IFI's archive collection that, given advances in molecular screening methodologies, can now be included in the genetic analyses. The comparison of archived (historical) and contemporary samples provides a

powerful approach to investigate both population structuring and temporal genetic stability. In addition to these juvenile brown trout, scales were also obtained from 362 older river brown trout (>16 cm in fork length & >1+ in age) during the same time periods. One additional archived sample set, comprising adult brown trout caught in 1974 at the mouth (lower reaches) of the Owenriff River (N=30), was also available for analyses. This sample set was considered as a component of the mixed adult brown trout lake stock and used to validate the river population genetic baselines identified from the genetic analyses (see below). Sampling details are provided in Table 1.

Table 1. Number and location of fish sampled from the main Lough Corrib tributary rivers (including historical sampling) used in this study (Ad – Adults (>1+), Ju – Juveniles (0+ & 1+)).

River	River Catchment	1974	1994		1996		1997		1998		2010	2012		2018		Total Ju	Total Ad	Total
		Ad	Ad	Ju	Ad	Ju	Ad	Ju	Ad	Ju	Ju	Ad	Ju	Ad	Ju			
Flaskagh	Clare						1	16	3	22						38	4	42
Clare	Clare		39	26				6								32	39	71
Kiltaclogher	Clare						5	8								8	5	13
Knocknagar	Clare							7								7	0	7
Grange	Clare		10		6	12	5	16	6	5		8	49			82	35	117
Abbert	Clare		14	7								23	36			43	37	80
Annacourta	Annacourta											2	46			46	2	48
Black River	Black River				27	17	2	4		4		1	48			73	30	103
Cross River	Cross River				1	19		21	1	10		7	41			91	9	100
Magheramore	Drimeeen						3	32								32	3	35
Drimneen	Drimeeen						5	20				5	43			63	10	73
Owenriff	Owenriff	30			141		1	49				6	42			91	178	269
Cornamona	Cornamona				11	46						6	40			86	17	103
Bealanabrack	Bealanabrack				3	41	1	5		8		2	42			96	6	102
Cong (River & Canal)	Cong										49	12	13	5	76	138	17	155
Total			63	33	189	135	23	184	10	49	49	72	400	5	76	926	392	1318

Lake Samples

In 2012, IFI undertook a fish stock assessment of L. Corrib (O’Grady & Delanty, 2013). As part of the fish sampling process, a set of scales were collected from 393 adult brown trout recorded for genetic analysis. Details regarding fish length, weight, and capture location were also recorded for each adult fish (See Fig. 3 for location of individual lake adult fish caught in the 2012 survey). Archived IFI scales from lake adult fish caught in 1996 (N=79) and 1997 (N=83), during pike surveys, were also available for analyses. It is important to note that sampling method employed for these historical surveys were different from the 2012 survey. Thus, brown trout caught on these historical sampling occasions were a by-product of the pike management programme carried out on the lake in 96 & 97. Although braided nets were used, mesh size was selected for pike, hence, brown trout caught during these surveys are

biased in terms of size and, contrary to the 2012 survey, do not fully represent the adult trout stock both in size range and distribution.

In additions to these, scales from adult ferox trout caught in L. Corrib in 2005 (N=16), 2006 (N=30), 2007 (N=30), and in Lough Mask in 2008 (N=25), 2009 (N=30) and 2010 (N=24), part of the Gargan *et al.* (2020) study, were also included in the analyses to assist with the identification of the source river populations within the L. Corrib catchment and for putative differences between L. Corrib and L. Mask ferox. “Ferox” trout were identified on the basis of morphology, especially their larger size (>38 cm in fork length) and coloration (Gargan *et al.*, 2020). Both random fertilised eggs (N=69) and adult broodstock (N=60) brown trout from the Oughterard Trout Hatchery, located on the Owenriff River, were also included in the analyses. This hatchery has been producing juveniles for stocking the L. Corrib catchment (tributary rivers and lake) for over 120 years. Adults taken from the lower reaches of the Owenriff River are used as broodstock for the hatchery. On average, 400 individuals (200 males and 200 females) are used per year. Fertilized eggs are kept in the hatchery and swim up (unfed) fry then released throughout the Owenriff system. Historically, these swim up fry were also released into various tributaries around the lake and directly into the lake itself. Given increasing evidence indicating that not all fish caught at the mouth of a river are part of the natural breeding population of that river, this broodstock sample (both fertilised eggs and adult broodstock) were treated as a mixture, with the null hypothesis that they should, in principle, assign to the Owenriff River population baseline. Considering the past stocking history of L. Corrib with Roscrea Hatchery derived fish, archived biopsy tissue material from adult brown trout from this hatchery (originally established with Leven brown trout and subsequently supplemented with local fish) were also included in the analyses (N=131) as the source baseline for the identification of Roscrea derived fish.

In summary, this study is based on the genetic screening of 1,318 river samples (adults, fertilised eggs and juveniles – Fig. 1), 710 lake caught adult trout (including 155 “ferox” trout) collected between 1996 and 2018 (Fig. 3), 129 brown trout (adults and fertilised eggs) from the Oughterard Hatchery, and 131 Roscrea adult farmed brown trout. Prior to commencement of laboratory sample processing for genetic analyses, all sampling information was transferred to an electronic database.

Genomic DNA was extracted using the Promega Wizard SV 96 genomic DNA purification system and transferred into 96 well microtiter plates for storage at -20°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 analyses of resulting genotypic data were only carried out on samples which had consistently amplified for a minimum of 70% of the marker loci (i.e., criteria for analyses).

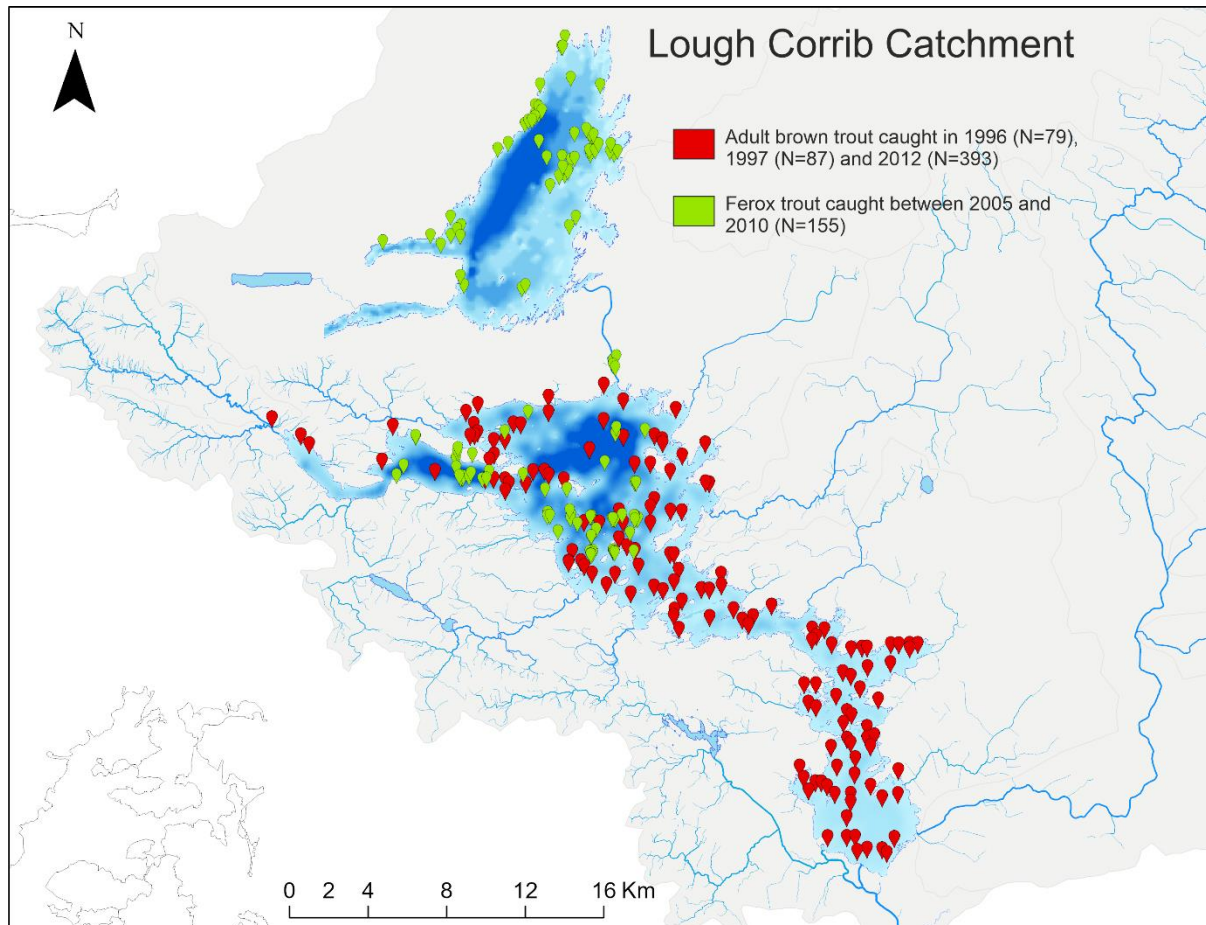


Figure 3. Lough Corrib river catchment indicating sampling locations for adult Eurasian trout examined in this study (red = location of adult brown trout caught between 1996 and 1997 and in 2012; green = location of known ferox trout caught between 2005 and 2010 from Gargan *et al.*, 2020).

4. Data Analyses and Results

In order to ensure an unbiased sample for the construction of the baseline, electrofishing site samples were checked for the presence of individuals belonging to the same family (i.e. full sibs) using COLONY v2.0.5.0 (Jones and Wang, 2010). This program tests whether locally collected samples are biased (i.e. dominated) by fish belonging to one or very few families. While a high incidence of full-sibs within samples can lead to biased assessment of population structuring, there was no evidence for the presence of large numbers of full-sibs within any of the river/stream samples in the whole data set. In the few instances, where full sibs were identified, no more than three individuals were present. Thus, there was no need to remove these from the analyses.

The program STRUCTURE v2.4 (Pritchard *et al.*, 2000) was used to investigate the impact(s) of brown trout stocking in L. Corrib, and to examine the patterns of both historical and contemporary population structuring. STRUCTURE clusters (i.e. groups) individuals based on their genetic similarities regardless of their origin. To ensure an unbiased sample data set for the examination of L. Corrib brown trout population structuring (i.e. definition of the baseline river populations), fish >1+ (any individual >16 cm in fork length) were excluded from this analysis, as these may represent transitory (migratory) older fish/adult fish. As outlined earlier, these river adults were used later, as part of the lake adult mixed stock, to validate 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 caught.

STRUCTURE analyses were carried out using a hierarchical approach that aimed to identify the major population groups within the data (i.e. potentially related by common ancestry) and the subsequent refinement of these down to single populations (i.e. genetic baselines). STRUCTURE was initially run with all samples (contemporary, historical and hatchery/farm) in a single analysis to investigate the potential differences in the genetic composition of samples from different locations over time resulting from the extensive stocking history of the lake. The hatchery sample (Roscrea) was used as the reference baseline for the identification of fish potentially derived from farm stocking in L. Corrib.

This first stage of the hierarchical STRUCTURE analysis indicates the presence of three distinct genetic clusters explaining the data (Fig. 4). The first cluster (green) is predominantly represented by samples from the Eastern side of the L. Corrib catchment (i.e. all samples from the River Clare catchment, Annacourta, Black, and Cross). The second cluster (red) comprises samples from the Western (i.e. Owenriff, Rusheeny, Cornamona and Bealanabrack) and Northeast areas (i.e. Cong) of the L. Corrib catchment. The Drimneen River, and its associated small tributary (Magheramore), appear as an area of interface between these two major genetic cluster groups. Within these two first genetically differentiated clusters, no statistically significant differences were noted between the historical and

contemporary samples. The third cluster (light yellow) consists of Roscrea brown trout. With few exceptions, there is no evidence of Roscrea derived fish among the L. Corrib samples. One of the juvenile brown trout caught in Grange in 1997, however, seems to be derived from Roscrea. There is also some suggestion of possible genetic introgression between Roscrea fish and those from the Drimneen River, caught in 1997. However, there is no evidence for introgression in the samples caught from the Drimneen River in 2012.

The Roscrea samples were then removed from subsequent STRUCTURE analyses that aimed to investigate patterns of population structuring, and to identify the main population(s) contributing to the lake adult trout stock. The archived historical samples, however, were retained in the analyses to examine possible temporal changes in the genetic composition of L. Corrib brown trout. Results from these analyses are summarised in graphical format in Fig. 5. At each iteration of the STRUCTURE analyses, the evaluation of the best number of clusters (K) explaining the genetic data offers valuable insight into the patterns of population genetic structuring of L. Corrib brown trout. Thus, there is clear evidence for further sub-structuring within both the Eastern and Western/Northeastern groups (see Fig 5 $K=3$ to $K=8$).

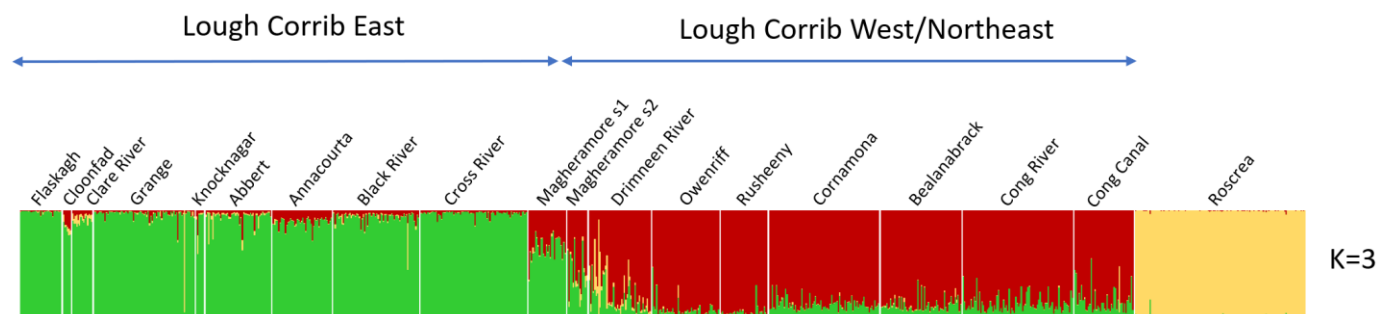


Figure 4. STRUCTURE Bar plots – First level population structuring of the Lough Corrib baseline river samples involving historical, contemporary and hatchery (Roscrea) derived samples. Where historical and contemporary samples were available from the same river, they were pooled together to simplify visualisation. Individual samples are represented as thin vertical coloured lines. In each case, different colours represent distinct genetic inferred lineages/clusters. Multi-coloured individual vertical lines are indicative of introgression and/or genetic similarities between inferred lineages, clusters and/or populations. Samples from distinct geographical locations are separated by thin white lines. In general, samples are displayed in an East-West geographical arrangement.

Within the Eastern group, there is a clear partition between samples from the Clare River catchment, and those from the Annacourta, Black and Cross rivers. While the samples from the last three rivers are ultimately genetically distinct (see $K=8$), they share a higher degree of genetic similarity to each other in comparison to the samples from the Clare River catchment. Samples from this river catchment

are further subdivided into three additional groups. The first is represented by samples from the Flaskagh and Cloonfad rivers, the second by samples from the Clare River (main channel) and, the third by samples from the Grange, Knocknagar and Abbert rivers. While some of these samples (e.g. Cloonfad, Clare and Knocknagar rivers) are represented by a comparatively small number of individuals, the fact that these samples are grouping in a geographically relevant context, adds support to the results.

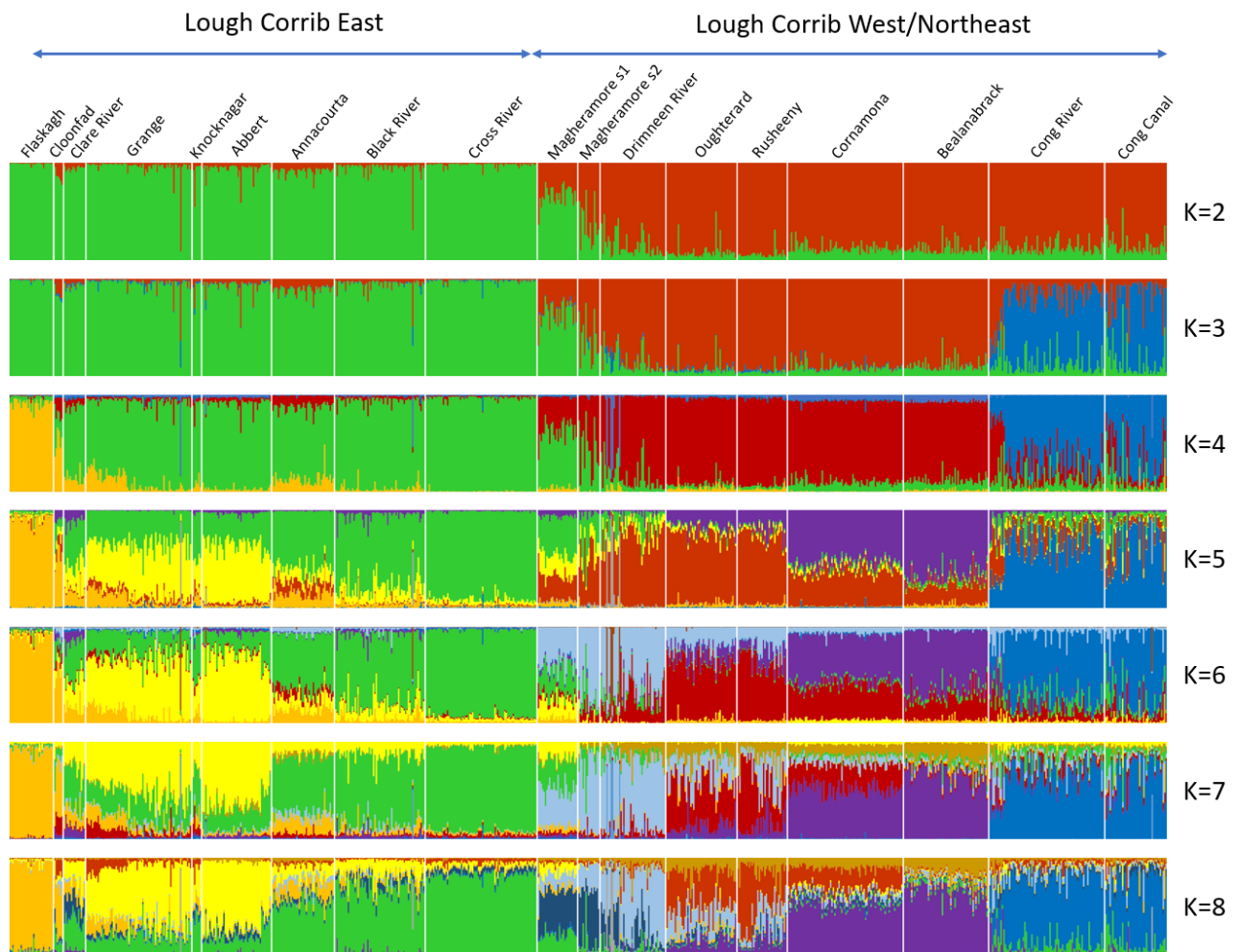


Figure 5. Resulting STRUCTURE bar plots exploring the best number of populations (K) explaining the genetic data. In this case, while there is evidence for further population substructuring, the genetic data was best explained by eight major groups (i.e. $K = 8$). Where historical and contemporary samples were available from the same river, they were pooled together to simplify visualisation. In each case (bar plot), individual samples are represented as thin vertical coloured lines. Different colours represent distinct genetic lineages/clusters/populations. Multi-coloured individual vertical lines are indicative of introgression and/or genetic similarities between inferred lineages/clusters/populations. Samples from distinct geographical locations are separated by thin white lines. In general, samples are displayed in an East-West geographical arrangement.

Within the Western/North-eastern cluster, samples from the Cong River are the first to break (Fig. 5 $K = 3$). The remaining western samples within this broader cluster, also form coherent genetic groups determined by geographical location. Thus, samples from Owenriff and Rusheeney (a small tributary from the Owenriff) form a single genetic cluster. While samples from both Cornamona and Bealanabrack rivers form their own genetic clusters, there is a substantial degree of genetic similarity between samples from these two geographically close rivers. As noted above, the Drimneen River catchment appears to be an interface zone between populations representing both the Western and Eastern groups. Within this comparatively small river catchment, there is evidence for the existence of two genetically distinct populations, one represented by samples from the main Drimneen River channel and one represented by samples from the Magheramore (a tributary of the Drimneen River). It is relevant to note, however, that the latter sample is represented by individuals caught in 1997 only. Thus, this observation needs to be confirmed. In support of the consistency of this result, however, is the fact that no genetic differences were detected between samples from the Drimneen River caught in 1997 and 2012. Indeed, in general, all archived historical juvenile samples collected over the 24 year period, covered by this study, clustered with their contemporary counterpart inferred populations, indicating the genetic stability of the genetic structuring observed among Lough Corrib brown trout populations over this time period.

While there is evidence for further population substructuring in some of the rivers, the data generated in this study, was best explained by eight major genetic groups or inferred populations that, thus, represent the current genetic baseline populations for the Lough Corrib catchment. These eight major genetic groups (and respective inferred populations) are basically defined by their geography and respective rivers of origin as follows: 1- Flaskagh and Cloonfad, 2- Clare Main Channel, 3 - Grange and Abbert, 4 - Annacourta, Cross, and Black, 5 - Cong River and Cong Canal, 6 - Cornamona and Bealanabrack), 7 – Owenriff, and 8 - Drimneen and Magheramore. Summary statistics (e.g. observed and expected heterozygosity, samples size, allele richness) for these groups / inferred populations are presented in Table 2.

Average allele richness values among inferred populations ranged from 4.4 (Flaskagh) to 6.3 (Roscrea Hatchery). The highest value observed for the latter is not surprising given the mixed nature of hatchery kept fish. Interestingly, excluding Roscrea, Drimneen was found to exhibit the highest level of allelic diversity. This inferred population was also characterised by a considerably higher incidence of private alleles (i.e. alleles restricted to single population). Notwithstanding this particularly interesting case, the overall levels of allele richness and both observed and expected heterozygosity were similar for all other samples.

Table 2. Summary statistics of inferred baseline populations identified in this study. The sample representing farm trout (Roscrea Hatchery) is included for comparison. N = no. of samples that have consistently amplified for over 70% of loci used for data analyses; Ar = average allelic richness; Private All = private (i.e. population specific) allele diversity; Ho and He = observed and expected heterozygosity. Please note that only inferred populations represented by more than 20 individuals are displayed.

Inferred Populations	River Catchment	Region	N	Ar	Private All	Ho	He
Flaskagh	Clare	1	37	4.4	-	0.518	0.542
Clare	Clare	2	25	5.1	-	0.552	0.581
Grange	Clare	3	75	5.7	0.05	0.597	0.620
Abbert	Clare	3	42	5.6	0.02	0.638	0.628
Annacourta	Annacourta	4	44	5.5	0.01	0.612	0.628
Black River	Black River	4	64	5.6	0.01	0.608	0.632
Cross River	Cross River	4	80	5.1	0.02	0.618	0.619
Cong River	Cong	5	78	5.1	0.03	0.562	0.599
Cong Canal	Cong	5	42	5.0	0.03	0.512	0.577
Cornamona	Cornamona	6	82	5.8	0.03	0.591	0.637
Bealanabrack	Bealanabrack	6	62	5.7	0.01	0.644	0.648
Owenriff	Owenriff	7	89	5.4	0.01	0.598	0.618
Magheramore	Drimneen	8	30	5.7	-	0.609	0.627
Drimneen	Drimneen	8	53	6.2	0.09	0.615	0.651
Roscrea Hatchery	Farm trout	-	123	6.3	0.11	0.638	0.679

The overall level of genetic divergence (F_{ST}) among the inferred L. Corrib brown trout populations was 0.052 (95% CI 0.048- 0.057), clearly demonstrating the existence of genetic divergence among them. Levels of genetic divergence between inferred populations (i.e. population pair-wise F_{ST} estimates) are displayed in Table 3. The lowest level of pair-wise inferred population divergence was observed between Grange and Abbert in the Clare River system (0.001 - 95% CI 0.001 – 0.007) in the east of L. Corrib, and between Cornamona and Bealanabrack (0.008 – 95%CI 0.002-0.015) in the west of the L. Corrib catchment. It is important to note that while there is statistically significant support for the genetic differences between these inferred populations, the differences are noticeably small in comparison to that observed between other inferred populations. Flaskagh (upper reaches of the Clare River catchment) and Cong Canal were the most divergent populations (Table 3). Overall, the inferred populations belonging to each of the two main initial clusters (i.e. East and West) identified from the STRUCTURE analyses (Fig. 4 K=2) were found to be more genetically similar to each other than with those belonging to the other main group.

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, which indicates lower levels”. All pair-wise population comparisons were found to be significant (i.e. 95%CI > 0).

Inferred Populations	Flaskagh	Clare	Grange	Abbert	Annacourta	Black River	Cross River	Cong River	Cong Canal	Cornamona	Bealanabrack	Owenriff	Magheramore	Drimneen
Flaskagh	-	(0.03-0.09)	(0.044-0.078)	(0.049-0.085)	(0.029-0.068)	(0.051-0.088)	(0.059-0.096)	(0.076-0.115)	(0.074-0.133)	(0.074-0.114)	(0.076-0.117)	(0.082-0.136)	(0.066-0.108)	(0.081-0.128)
Clare	0.056	-	(0.001-0.046)	(0.001-0.056)	(0.005-0.054)	(0.001-0.044)	(0.007-0.053)	(0.048-0.099)	(0.04-0.109)	(0.024-0.073)	(0.027-0.073)	(0.028-0.082)	(0.003-0.059)	(0.037-0.089)
Grange	0.059	0.019	-	(0.001-0.007)	(0.005-0.023)	(0.004-0.017)	(0.01-0.024)	(0.042-0.063)	(0.045-0.091)	(0.037-0.055)	(0.046-0.071)	(0.035-0.061)	(0.021-0.044)	(0.042-0.068)
Abbert	0.066	0.023	0.001	-	(0.008-0.025)	(0.001-0.016)	(0.013-0.027)	(0.037-0.057)	(0.047-0.092)	(0.036-0.055)	(0.045-0.07)	(0.041-0.07)	(0.019-0.043)	(0.041-0.069)
Annacourta	0.047	0.025	0.013	0.015	-	(0.002-0.02)	(0.003-0.019)	(0.038-0.061)	(0.042-0.098)	(0.029-0.047)	(0.038-0.062)	(0.034-0.065)	(0.018-0.042)	(0.034-0.06)
Black River	0.068	0.015	0.009	0.008	0.010	-	(-0.001-0.01)	(0.038-0.059)	(0.046-0.101)	(0.031-0.05)	(0.035-0.057)	(0.034-0.062)	(0.017-0.039)	(0.033-0.06)
Cross River	0.077	0.026	0.017	0.020	0.009	0.004	-	(0.041-0.061)	(0.047-0.097)	(0.03-0.047)	(0.034-0.057)	(0.037-0.063)	(0.016-0.037)	(0.034-0.06)
Cong River	0.094	0.070	0.052	0.046	0.049	0.049	0.051	-	(0.02-0.056)	(0.03-0.05)	(0.038-0.061)	(0.025-0.052)	(0.04-0.067)	(0.026-0.047)
Cong Canal	0.102	0.070	0.066	0.069	0.068	0.072	0.071	0.036	-	(0.043-0.088)	(0.049-0.098)	(0.041-0.09)	(0.05-0.108)	(0.042-0.092)
Cornamona	0.093	0.046	0.046	0.045	0.038	0.040	0.038	0.040	0.065	-	(0.002-0.015)	(0.008-0.033)	(0.021-0.04)	(0.014-0.032)
Bealanabrack	0.096	0.047	0.058	0.057	0.050	0.046	0.045	0.048	0.071	0.008	-	(0.015-0.043)	(0.03-0.052)	(0.02-0.041)
Owenriff	0.108	0.052	0.047	0.054	0.048	0.047	0.0481	0.0369	0.0633	0.0188	0.0276	-	(0.022-0.055)	(0.008-0.035)
Magheramore	0.086	0.027	0.032	0.031	0.028	0.027	0.0255	0.0531	0.0768	0.03	0.0407	0.0361	-	(0.024-0.052)
Drimneen	0.104	0.060	0.054	0.055	0.047	0.046	0.046	0.035	0.065	0.023	0.0292	0.020	0.037	-

To further examine the genetic relationships among the inferred brown trout populations within the L. Corrib catchment (and also as an additional confirmation for the results of the STRUCTURE analyses), a neighbour-joining (NJ) phylogenetic tree, based on Nei's DA (Nei *et al.*, 1983), was constructed using POPTREE2 (Takezaki *et al.*, 2010). The results of this analysis (Fig. 6), unambiguously confirms the presence of the two major geographical groups identified in the STRUCTURE analysis (East and West), the third main group (Cong), and also the overall relationships among the inferred populations that largely follow geography.

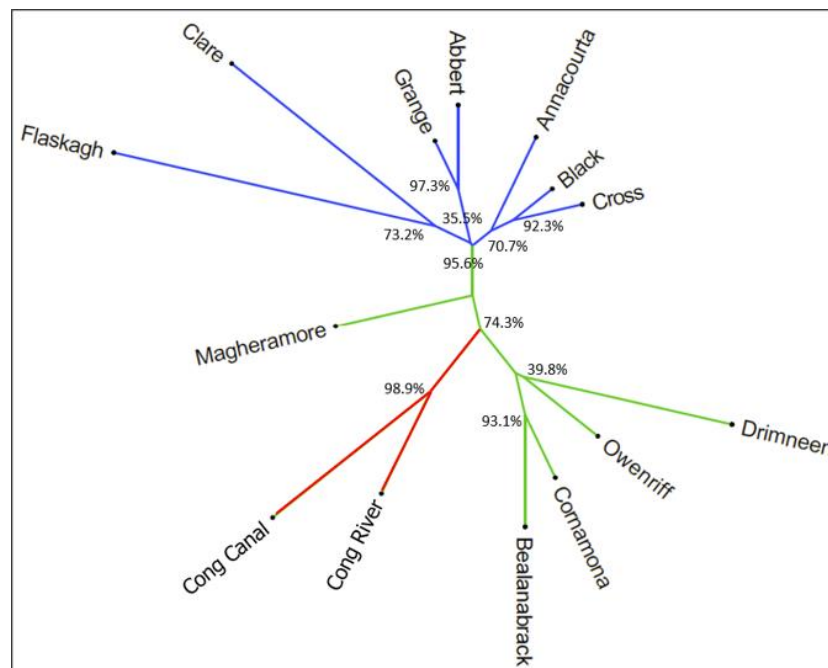


Figure 6. Unrooted NJ phylogenetic tree based on Nei's DA (1983) genetic distance illustrating the relationship among Lough Corrib inferred populations. Different colours represent major genetic groups as identified and coded in the STRUCTURE analysis (East and West). Percentage bootstrap support is shown at nodes.

The function *divMigrate* from the *diversity* R package (Keenan *et al.*, 2013) was used to examine patterns and levels of gene flow between the inferred populations. This function estimates the relative migrations rates (Nm) and direction of gene flow between and among populations. Results from this analysis (Fig. 7) provide further validation for previous findings (STRUCTURE, NJ and F_{st}) regarding the genetic relationship among the inferred populations. Thus, populations belonging to each of the two main groups (East and West) are characterised by higher levels of gene flow in comparison to populations from the other group. Interestingly, evidence seems to suggest only limited and asymmetric (i.e. from west to east) gene flow among populations belonging to these two main groups.

Within each group, there are clear patterns of increased levels of gene flow among populations, in particularly those which are geographically close. For instance, within the eastern group, it is clear that Abbert and Grange share a higher level of bi-directional gene flow with each other than with those in the group comprising Annacourta, Black and Cross. These latter populations are again characterised by higher levels of bidirectional gene flow among themselves but with a bias between Black and Cross (more geographically close) in comparison to Annacourta.

Similar patterns and (distinct) levels of gene flow are also noted among populations of the western group. Thus, it is clear that there is a substantial level of bidirectional gene flow between Cornamona and Bealanabrack, and to a lesser extend between those two populations and Owenriff. Within the western group, Cong is a clear outgroup with limited gene flow with other populations of the group. It is interesting to note, however, the asymmetric gene flow pattern between Cong Canal and Cong River and the evidence for restricted gene flow between Cong River and Owenriff. It is also important to note that Cong River and Cong Canal are genetically distinct populations. In the eastern group, Flaskagh seems be completely isolated (no gene flow) from other populations of the group.

The power (i.e. usefulness) of the inferred baseline river populations for individual assignment (i.e. to be able to assign a lake caught adult fish to the correct baseline) was assessed using the leave-one out test implemented in ONCOR (Kalinowski *et al.*, 2007). Given the high level of genetic similarities observed between/among several populations (e.g., Abbert and Grange; Cornamona and Bealanabrack; Cross, Black and Annacourta), which is supported by high levels of gene flow between/among them, results are presented in Table 4 for regional reporting groups (RG) as follows: RG01 – Flaskagh; RG02 - Clare; RG03 - Abbert and Grange; RG04 – Cross, Black and Annacourta; RG05 – Cong River; RG06 – Cong Canal; RG07 – Cornamona and Bealanabrack; RG08 – Owenriff; RG09 – Magheramore; RG10 – Drimneen. From both conservation and management perspectives, this approach is biologically more realistic, reflecting the high level of natural straying between/among certain geographically close rivers/populations. Thus, it makes little sense to treat some of these as completely independent populations.

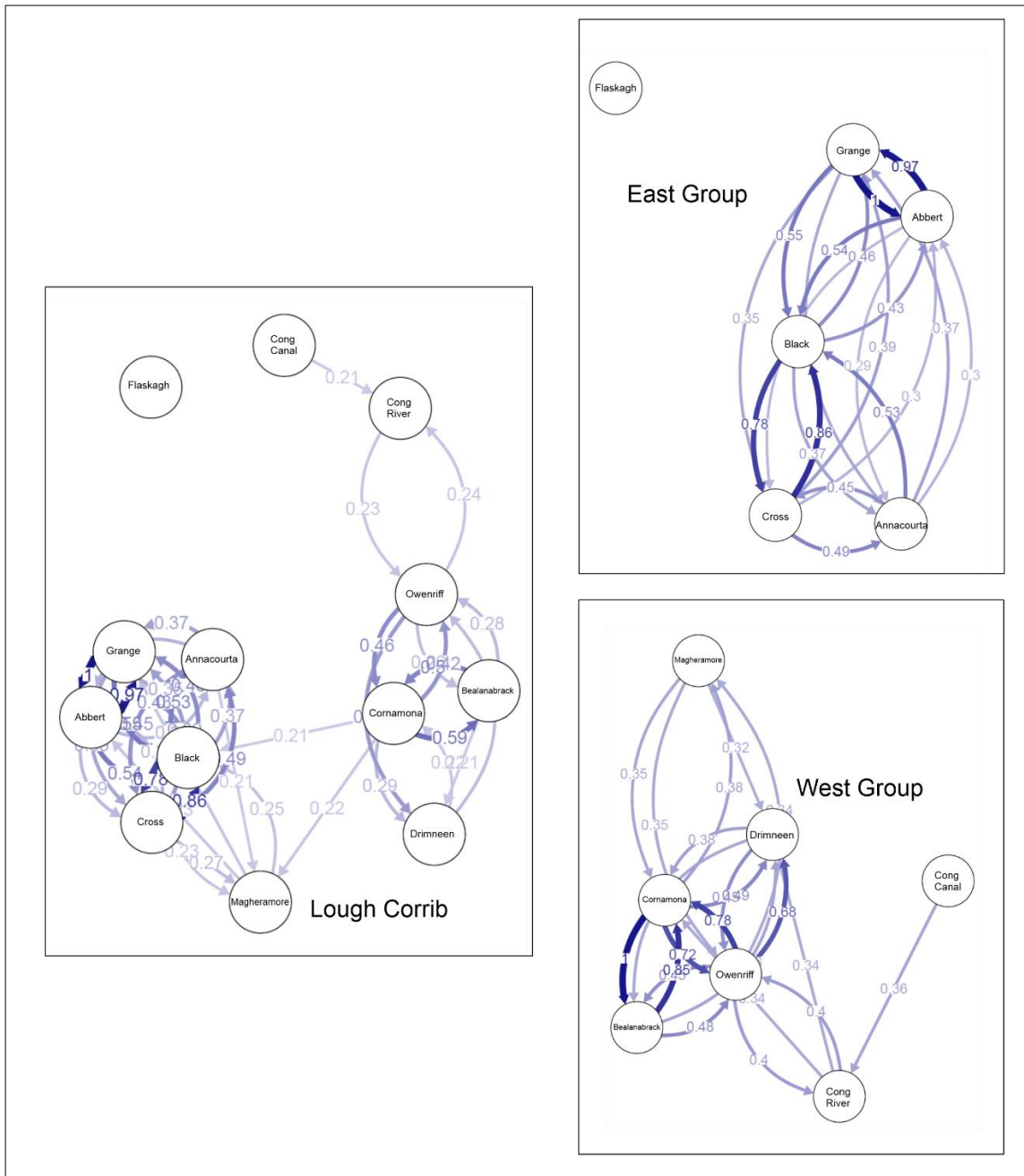


Figure 7. Networks illustrating the relative rate of migration (Nm) among inferred populations for the whole data set (Lough Corrib) and for the two main population groups (West and East) identified from the STRUCTURE and NJ analyses. The proximity of nodes (populations) indicates more gene flow among them than with others; arrows indicate direction and levels of migration between and among populations with darker and thicker blue representing higher migration rates than lighter (thinner) blue.

Table 4. Summary results (in %), of correct self-assignment (ONCOR) to inferred Regional Reporting Groups (diagonal values), including miss-assigned individuals. The broader geographical regions (East or West) for the ten Regional Reporting Groups are also provided for reference. The darker colour in the green heat-map scale indicates stronger assignment levels.

Regional Reporting Group		Lough Corrib - East				Lough Corrib - West/Northeast						Farm
		RG01	RG02	RG03	RG04	RG05	RG06	RG07	RG08	RG09	RG10	
Flaskagh	RG01	89%	5%	-	5%	-	-	-	-	-	-	-
Clare	RG02	-	33%	33%	33%	-	-	-	-	-	-	-
Grange/Abbert	RG03	1%	2%	79%	13%	-	-	1%	4%	2%	-	-
Cross/Black/Annacourta	RG04	2%	3%	7%	80%	1%	1%	1%	2%	4%	1%	-
Cong River	RG05	-	-	1%	1%	90%	7%	-	-	-	-	-
Cong Canal	RG06	3%	6%	-	9%	-	64%	6%	9%	-	3%	-
Cornamona/Bealanabrack	RG07	-	-	1%	3%	1%	-	79%	9%	4%	2%	-
Owenriff	RG08	-	-	-	5%	2%	-	8%	82%	2%	3%	-
Magheramore	RG09	-	-	5%	13%	3%	-	5%	3%	71%	-	-
Drimneen	RG10	-	-	3%	3%	-	-	13%	10%	18%	54%	-
Farm	Farm	-	-	-	-	-	-	-	-	-	-	100%

The power to correctly identify individuals to reporting regional groups ranged from 33% (Clare) to 100% (Roscrea farm trout) with an average value of 75%. Self-assignment values were greater than 70% for eight of the 11 reporting groups (including farm), confirming the convenience of these population reporting groups as “baselines” for the identification of the origins of L. Corrib adult fish (i.e. individual genetic assignment). The low self-assignment for the RG02 (Clare) is not surprising given the comparatively small sample size for this particular sample (N=22). Nevertheless, it is relevant to note that misassignments were predominantly linked to other geographically close RGs and, thus, belonging to the same broad geographical group (i.e., East and West). For instance, the majority of misassigned individuals from RG03 (Grange/Abbert) assign to RG01, RG02 or RG04 representing geographically close populations (all belonging to the East broad group). This was also often the case for the other reporting groups (Table 4). It is also important to emphasise that no misassignments were noted for the Roscrea (farm) group, thus, confirming the highly divergent genetic status of fish of farm origin, and providing further validation to assignments to this particular “baseline” reporting group. That is, the data allows the unambiguous identification of individuals from farm (Roscrea) origin.

Assignments of adult samples caught both in the lake and rivers (for river this included fish >16cm), in addition to, samples representing the Oughterard Hatchery (fertilised eggs and adult broodstock) to the identified genetic baseline populations were also carried out with ONCOR. Only fish with an assignment probabilistic value (P) of 0.7 or higher were considered to be biologically robust assignments. Summary results for these analyses are presented in Table 5 (Rivers and Oughterard Hatchery), Table 6 (lake caught fish) and Table 7 (ferox).

Table 5. Summary results (both in absolute numbers and percentages), of individual assignment (ONCOR) of both river adults, and Oughterard Hatchery (fertilised eggs and adult broodstock) to regional reporting groups consisting of single or group of populations. Grey shaded cells in the table indicate agreement between river of capture and regional reporting group. Regional Reporting Groups are as follows: RG01 - Flaskagh, RG02 - Clare; RG03 - Abbert and Grange; RG04 – Cross, Black and Annacourta; RG05 – Cong River; RG06 – Cong Canal; RG07 – Cornamona and Bealanabrack; RG08 – Owenriff; RG09 – Magheramore; RG10 – Drimneen. The broader geographical regions (East or West/Northeast) for the ten Regional Reporting Groups are also provided for reference.

Origin of adult fish	Reporting Group Assignment of River Caught fish										Farm
	Lough Corrib - East				Lough Corrib - West/Northeast						
	RG01	RG02	RG03	RG04	RG05	RG06	RG07	RG08	RG09	RG10	
Flaskagh	3 (100%)	-	-	-	-	-	-	-	-	-	-
Clare	4 (15.4%)	10 (38.5%)	10 (38.5%)	2 (7.7%)	-	-	-	-	-	-	-
Kiltaclogher	-	-	3 (100%)	-	-	-	-	-	-	-	-
Grange	2 (9.5%)	2 (9.5%)	13 (61.9%)	3 (14.3%)	-	-	1 (4.8%)	-	-	-	-
Abbert	-	-	26 (92.9%)	-	-	-	1 (3.6%)	1 (3.6%)	-	-	-
Annacourta	-	-	-	2 (100%)	-	-	-	-	-	-	-
Black	-	-	2 (13.3%)	12 (80%)	-	-	-	1 (6.7%)	-	-	-
Cross	-	-	-	4 (80%)	-	-	-	-	1 (20%)	-	-
Magheramore	-	-	-	1 (50%)	-	-	-	-	1 (50%)	-	-
Drimneen	-	-	-	-	-	-	1 (12.5%)	1 (12.5%)	-	6 (75%)	-
Owenriff	1 (1%)	-	2 (1.9%)	6 (5.8%)	8 (7.8%)	-	41 (39.8%)	39 (37.9%)	-	5 (4.9%)	1 (1%)
Cornamona	-	-	-	-	-	-	15 (100%)	-	-	-	-
Bealanabrack	-	-	-	-	-	-	4 (100%)	-	-	-	-
Cong River	1 (33.3%)	-	-	-	1 (33.3%)	-	-	1 (33.3%)	-	-	-
Cong Canal	-	-	-	2 (40%)	2 (40%)	-	-	1 (20%)	-	-	-
Hatchery adults	-	-	1 (2.2%)	-	28 (60.9%)	-	6 (13%)	11 (23.9%)	-	-	-
Hatchery eggs	1 (2.3%)	-	2 (4.7%)	-	5 (11.6%)	-	18 (41.9%)	13 (30.2%)	2 (4.7%)	2 (4.7%)	-

Of the 500 specimens, representing both the river adults and Oughterard Hatchery that met criteria for analyses, 332 (~66.5%) assigned with probability criteria (P) larger than 0.7 to one of the inferred baseline populations (Table 5). Overall, there was a clear correlation between river origin and assignment. Thus, river adult fish tend to assign to a reporting group representing the population, or group of populations, which make up for the local regional reporting group (see grey shaded cells in Table 5). Where there were discrepancies between river of capture and regional reporting group, adult fish tend to assign to another geographically close reporting group, again with noticeable bias towards the broad L. Corrib geographical groups (East and West). For instance, all adults taken from both Cornamona ($N=15$) and Bealanabrack ($N=4$) assigned to the local regional reporting group RG07 (Cornamona and Bealanabrack). For the Owenriff, however, only ~38% of the adult caught fish ($N=103$) assigned to RG08, while the larger proportion (~40%) assign to geographically close RG07 (Cornamona and Bealanabrack). Similarly, the majority of the adults caught both in Abbert ($N=28$) and Grange ($N=21$) assign to RG03 (Abbert and Grange). These assignment results provide an empirical validation for the usefulness of the genetic baselines (population reporting groups) in identifying the source of L. Corrib adult fish. It is also interesting to report that despite not meeting the probability criteria (i.e. $P < 0.7$), the patterns of assignment of these river caught fish are similar to those that

have met the criteria (i.e. $P > 0.7$). That is, in general, these fish tend to assign to either the same river and/or broader geographical groups from where they were originally caught.

The Oughterard Hatchery samples (both fertilised eggs and adult broodstock) were found to comprise of individuals from different origins. For the fertilized eggs, RG07 derived fish (Cornamona and Bealanabrack) represented the larger fraction of the original broodstock (~42%) followed by Owenriff fish (~30%). There was also evidence of contribution from the Cong River (~12%). For the adult hatchery broodstock, a large proportion consisted of RG05 fish (Cong River - ~61%), followed by RG08 (Owenriff - ~24%) and RG07 (Cornamona and Bealanabrack - ~13%). It is also interesting to note that only one adult fish, caught in 1996, from Owenriff assigned to the Roscrea (farm) group.

A total of 653 (92%) adult lake caught fish (including ferox) met criteria for subsequent analyses. Of these, 504 (77.2%) assigned with $P > 0.7$ to one of the inferred baseline populations (Table 6). Excluding known ferox, overall (i.e., taking into consideration historical and contemporary samples), the main contributors for the L. Corrib adult brown trout mixed stock are fish from RG03 (Abbott and Grange – ~21%), RG07 (Cornamona and Bealanabrack - ~13%), RG05 (Cong River - ~11%), RG08 (Owenriff - ~7%) and RG04 (Cross, Black and Annacourta – ~6%). In comparison, contributions from the remaining reporting groups are much reduced (Table 6). There was no evidence for the presence of fish from either a farm (Roscrea) origin or RG01 and RG02 (representing the upper reaches of the Clare River catchment) in the data set. Interestingly, the regional reporting groups comprising the two broad L. Corrib regions contribute similar proportions to the brown trout lake mixed stock with ~27% and ~36% overall for the reporting groups from the East and West/Northeast groups respectively (Table 6). It is also interesting to note that, notwithstanding differences in the sampling design for lake samples caught in 1996 and 1997, and in 2012 (i.e. allowing for possible bias for samples caught in 1996 and 1997), there is a general consistency in the results regarding the contribution of the different regional groups, thus providing support for the results. One significant and intriguing result from this analysis is that, despite being a comparatively smaller river, the Cong River appears as a significant contributor (~11%) to the L. Corrib adult mixed stock (Table 6). The percentage of unassigned adult lake fish (i.e., $P < 0.7$) was consistent over time with an average of 36.5% overall (Table 6). Again, it is relevant to report that identical analyses including all caught lake fish, regardless of the assignment criteria (i.e., including all individuals with $P > 0.7$ and $P < 0.7$), largely show similar results in terms of river and/or regional group contribution to the lake stock.

For the known ferox trout caught in L. Corrib (from Gargan *et al.*, 2020), ~95.6% (66) primarily assigned to Cong River with the remaining fish assigning to Cong Canal (~2.8%) and Drimneen (~1.4%) (Table 7). For the ferox trout caught in L. Mask, ~84% (61) assigned to Cong Canal while the remaining fish assigned to the Cong River (~16%). No assignments of these ferox fish were noted for any of the other

existing L. Corrib reporting groups. Similar to that noted for the L. Corrib adult trout caught (Table 6), in both instances, there was good consistence for assignments over multiple years, thus, validating the results of these assignments.

Table 6. Summary results (both in absolute numbers A and percentages B), of individual assignment (ONCOR) of lake caught adults to regional reporting groups. The darker colour in green heat-map scale indicates stronger assignments. Regional Reporting Groups are as follows: RG01 - Flaskagh, RG02 - Clare; RG03 - Abbert and Grange; RG04 – Cross, Black and Annacourta; RG05 – Cong River; RG06 – Cong Canal; RG07 – Cornamona and Bealanabrack; RG08 – Owenriff; RG09 – Magheramore; RG10 – Drimneen. The broader geographical regions (East or West/Northeast) for the ten Regional Reporting Groups are also provided for reference. Unassigned adult fish (i.e. $P < 0.7$) are also reported.

A)

Lake caught fish assigning to:	1996	1997	2012	Total
Lough Corrib East				
RG01 (Flaskagh)	-	-	-	-
RG02 (Clare)	1	-	-	1
RG03 (Abbert/Grange)	8	3	96	107
RG04 (Cross/Black/Annacourta)	5	4	20	29
Lough Corrib West/Northeast				
RG05 (Cong River)	7	12	36	55
RG06 (Cong Canal)	2	7	8	17
RG07 (Cornamona/Bealanabrack)	14	10	43	67
RG08 (Owenriff)	2	9	23	34
RG09 (Magheramore)	2	1	5	8
RG10 (Drimneen)	1	-	1	2
Unassigned ($P < 0.7$)	31	28	125	184
Farm	-	-	-	-
Total	42	46	232	504

B)

Lake caught fish assigning to:	1996	1997	2012	Overall
Lough Corrib East				
RG01 (Flaskagh)	-	-	-	-
RG02 (Clare)	1.4%	-	-	0.2%
RG03 (Abbert/Grange)	11.0%	4.1%	26.9%	21.2%
RG04 (Cross/Black/Annacourta)	6.8%	5.4%	5.6%	5.8%
Lough Corrib West/Northeast				
RG05 (Cong River)	9.6%	16.2%	10.1%	10.9%
RG06 (Cong Canal)	2.7%	9.5%	2.2%	3.4%
RG07 (Cornamona/Bealanabrack)	19.2%	13.5%	12.0%	13.3%
RG08 (Owenriff)	2.7%	12.2%	6.4%	6.7%
RG09 (Magheramore)	2.7%	1.4%	1.4%	1.6%
RG10 (Drimneen)	1.4%	-	0.3%	0.4%
Unassigned ($P < 0.7$)	42.5%	37.8%	35.0%	36.5%
Farm	-	-	-	-

Table 7. Summary results (numbers) of individual assignment (ONCOR) of ferox trout caught both in L. Corrib and L. Mask to inferred river baseline populations.

L. Corrib Ferox assigning to:	2005	2006	2007	-	-	-	Total
Cong River	14	27	25	-	-	-	66
Cong Canal	-	1	1	-	-	-	2
Drimneen	-	-	1	-	-	-	1
L. Corrib Mask assigning to:	-	-	-	2008	2009	2010	Total
Cong River	-	-	-	4	-	8	12
Cong Canal	-	-	-	20	26	15	61

To further understand the distribution of ferox trout in L. Corrib, the individual location of known ferox trout (caught in 2006 and in 2007 as reported in Gargan *et al.*, 2020) was examined against adult trout caught in the 2012 lake survey. Results of this comparison, summarised in Fig. 9, indicates a remarkable agreement in the distribution of these fish in L. Corrib, again supporting the conclusion that the adult fish caught in 1996 and 1997, and also in the 2012 survey are ferox trout. Further support for this conclusion is the fact that the average length of these fish assigning to “Cong River” was 43.6 cm, which fits the size criteria for ferox trout. For comparison, the average length for all the other lake caught adult assigning to the other reporting regions was 38.4 cm.

Three significant conclusions that can be drawn from these combined results (Tables 6 and 7 and Fig. 8 and Fig. 9) are that: 1) L. Corrib ferox trout seems to be genetically related to the Cong River; 2) A significant proportion of the L. Corrib adult stock (varying from 9.6% to 16.2%) seem to be generally linked to the Cong trout and, hence, appear to be ferox, and 3) ferox trout from L. Corrib and L. Mask are genetically distinct. The first observation was not surprising given that ferox trout has been previously associated with the Cong system (Gargan *et al.*, 2020). The second observation, however is unexpected, particularly because of the small spawning carrying capacity of the Cong River (1.6 km in length). The third observation confirms limited gene flow between L. Mask and L. Corrib ferox. These findings are further discussed in the summary session.

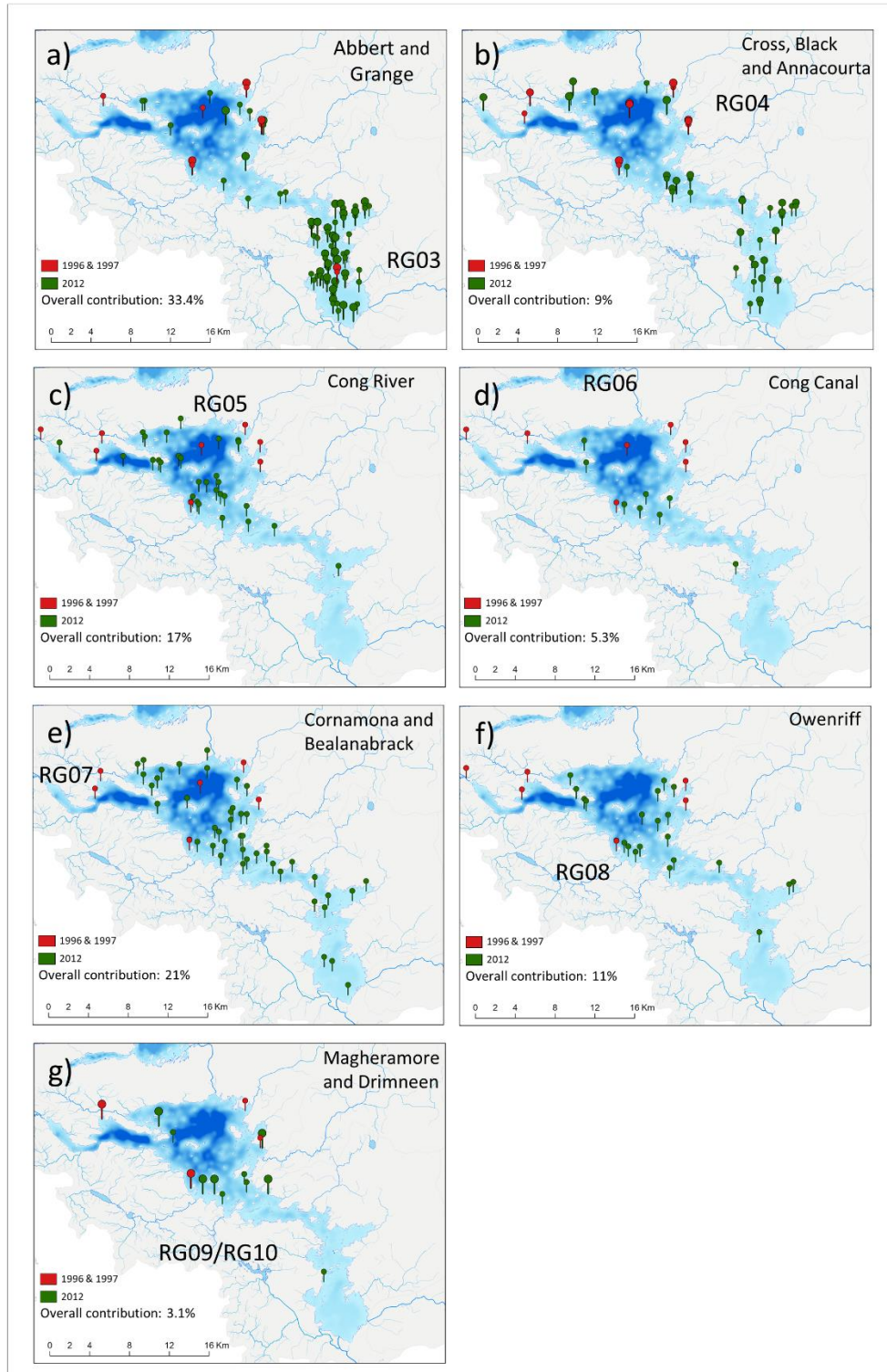


Figure 8. Individual assignment of lake caught adult fish taking into consideration the specific location of capture. Assignments refer to reporting groups as follows: RG03 - Abbert and Grange; RG4 – Cross, Black and Annacourta; RG05 – Cong River; RG06 – Cong Canal; RG07 – Cornamona and Bealanabrack; RG08 – Owenriff; RG09/RG10 – Drimneen/Magheramore. Reporting regions with contribution less than 5% are not shown. The position of the RG labels on map coincides with river locations.

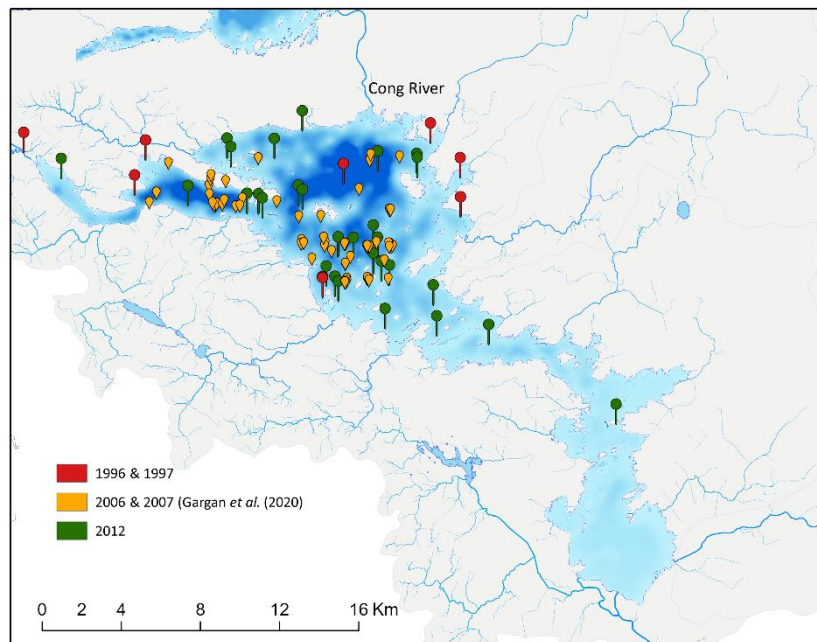


Figure 9. Individual assignment of lake caught (in 1996 and 1997 – “red”, and in the 2012 – “dark green) adult, taking into consideration the specific location of capture, to the Cong River (RG05). Also displayed are the known ferox trout caught in 2006 and 2007.

5. Summary

Lough Corrib is well known for its brown trout populations, which include the long-lived, late maturing, piscivorous and highly prized ferox trout (*Salmo ferox*). Over the past two centuries, L. Corrib and its tributary rivers have been subjected to several environmental disturbances that have contributed to the alteration of the natural lake environment and the loss and/or fragmentation of suitable spawning and nursery areas for wild brown trout. These have included urban growth and associated discharges, arterial drainage of river networks, farming activities and agricultural run-off, introduction of alien species and stocking. There is now compelling evidence from scientific literature showing that all these factors can adversely impact population substructuring, genetic diversity and, ultimately long-term sustainability of populations. The results presented in this study, however, suggests a noteworthy level of natural resilience from the wild L. Corrib brown trout, confirming what has been found in other similar investigations focusing on lake systems impacted by human mediated activities (L. Sheelin - Delanty *et al.*, 2019).

L. Corrib population genetic structuring and connectivity:-

Eurasian (brown) trout inhabiting L. Corrib, and its river catchment, are represented by several genetically distinct populations that were found to be genetically stable over a period of at least 24 years. Of particular relevance is that these populations are clearly partitioned into two broad geographical groups (East and West/Northeast). The eastern group is represented by samples from the Clare River and its tributaries (Flaskagh, Clare, Kiltaclogher, Knocknagar, Grange, Abbert) and from the Annacourta, Black and Cross rivers. The western/North-eastern group comprises samples from Magheramore, Drimneen, Owenriff, Cornamona and Bealanabrack and Cong. Within each of these two main groups, there is clear evidence of varying levels of population connectivity (i.e., gene flow).

Thus, in the eastern group, there is evidence for considerable levels of bidirectional gene flow (natural fish straying) between Abbert and Grange, and also among Cross, Black and Annacourta. Nevertheless, gene flow from Flaskagh to other populations in the Clare River catchment seems to be non-existent or very limited. In the western/north-eastern group, there is clear evidence for natural gene flow between Cornamona and Bealanabrack, and also between these two populations and Owenriff. Similarly, despite significant genetic differences, there is clear evidence of gene flow between Drimneen and Magheramore. Within the western/north-eastern group, the two brown trout populations identified in Cong (Cong River and Cong Canal) are substantially distinct from other populations of this group (i.e. West). They are also significantly genetically distinct from each other.

The patterns and levels of population divergence among populations within the L. Corrib catchment likely reflect the combined effects of divergence in isolation resulting both from the relatively recent glacial history of Ireland, and human mediated changes in the habitat over the past 150 years. In particular, through the creation of surface channels from previously subterranean sections of some rivers flowing into the lake (i.e., Cong Canal and Clare River) and, removal of natural barriers to movement (i.e., Clare River, Owenriff falls (blasted in 1958) and Failmore falls (blasted in 1957)).

Results also suggest the existence of a potential correlation between geological type (i.e. acid versus limestone underlying geology) and the geographical distribution of populations belonging to two broad geographical groups (Fig 10). Thus, the west and the east of the L. Corrib catchment are characterised by acidic and alkaline (limestone) soils respectively, which in turn would have an effect on the water chemistry (water pH). Other brown trout genetic based studies (Prodöhl *et al.*, 2019) have suggested that water pH can be a strong driver for local adaptation. The existence of such genetic structuring within L. Corrib catchment is, thus, linked to a complex evolutionary history, leading to

important adaptive differences among populations that contributes to their productivity and resilience and, hence, merits special attention from both management and conservation viewpoints.

The levels and patterns of population genetic divergence reported in the present study are generally in agreement with the previous study by Massa-Galluci *et al.* (2010), but with one important difference. In the Massa-Galluci *et al.* (2010) study, the Grange sample was found to be genetically highly divergent (several orders of magnitude) from all other samples within the Lough Corrib catchment. The present study, however, do not support this previous result. Thus, while genetically distinct, brown trout from Grange were found to be very similar to Abbert brown trout. This finding was corroborated through use of multiple genetic statistical analyses (e.g., STRUCTURE, NJ, Gene flow) and screening of both historical and contemporary samples.

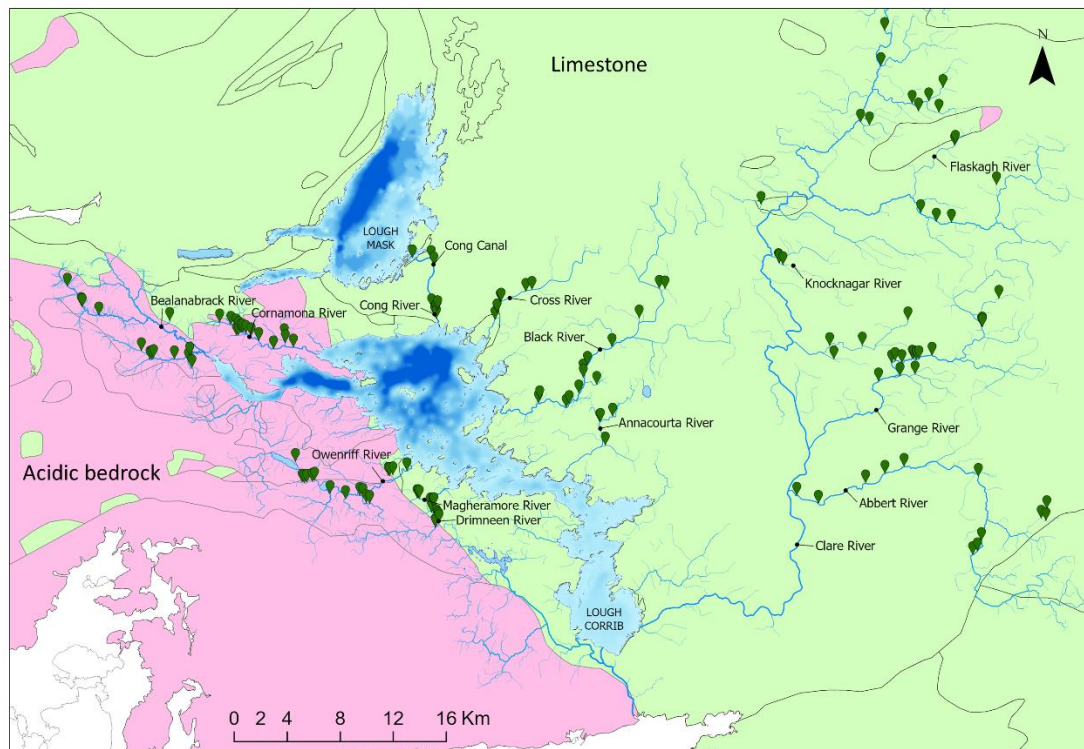


Figure 10. Lough Corrib river catchment (area of study) including river sampling locations (dark pins). Different colours represent distinct soil composition (green – alkali; pink – acidic).

A review of the results described by Massa-Galluci *et al.* (2010) provides important clues to explain the discrepancy of the findings in both surveys. In particular, the presence of alleles among Grange samples that were absent in all other L. Corrib samples examined in that study. Subsequent re-examination of these alleles, carried out as part of the current study, however, identified them as

typical of Atlantic salmon (*Salmo salar*), and not of brown trout. Hence, the Grange sample from Massa-Galluci *et al.* (2010)'s study was in fact mostly represented by Atlantic salmon. These findings have compromised some of the results reported in Massa-Galluci *et al.* (2010)'s study, in particular the potential contribution of different baseline rivers to the Lough Corrib mixed trout population. This is now re-assessed in this study.

GSI to identify the main brown trout populations, and associated tributary rivers, contributing to the lake adult mixed brown trout stock

As a consequence of the high level of genetic similarities observed between/among several populations (e.g., Abbert and Grange; Cornamona and Bealanabrack; Cross, Black and Annacourta), from a management conservation perspective, the contributing populations to the mixed lake stock were provided in terms of reporting groups. These consisted of one or more populations, depending on the level of genetic connectivity and, hence, divergence among them. Taking into consideration adult lake samples collected in 2012, which involved a sampling design that allows for accurate representation of the L. Corrib adult brown trout stock, the main contributors to the lake stock were Abbert and Grange (~27%), Cornamona and Bealanabrack (~12%), Cong River (~10%), Owenriff (~6%) and Cross, Black and Annacourta (~6%). Notwithstanding differences in the sampling design for historical samples (1996 and 1997), it is relevant to note that these proportions are consistent over a period of at least 16 years.

The overall proportion (36.7%) of unassigned adult lake fish (i.e., $P < 0.7$) needs to be considered with caution. First, it is important to note that this estimate likely represents an overestimation of the true value, as many individuals with assignment values of $P > 0.6$ are, in fact, assigning to the correct baseline. It is also important to note that fish that cannot be assigned, with great certainty (i.e., $P > 0.7$), to a baseline sample also reflects natural 'straying' of adults from different rivers with subsequent introgression (i.e., first generation hybrid individuals will often display low P values reflecting a mixture). This hypothesis is supported by the patterns and levels of gene flow observed between and among samples in this study. There is also the possibility that it may reflect hatchery impacts, albeit considered minimal. Missing baseline populations is another important factor that can account for the presence of unassigned adult lake fish (i.e., with low assignment confidence). While tributaries included in this study were well known trout rivers, not all tributaries were sampled. Indeed a small number of moderate sized sub-catchments (e.g., Lough Kip, Owenwee/Fallomer, Cregg), and numerous smaller tributaries (as many as 10 to 15) that run directly into L. Corrib were not included

as part of the 2012 study. Future surveys including these and other unsampled areas within Lough Corrib would help to clarify this particular question.

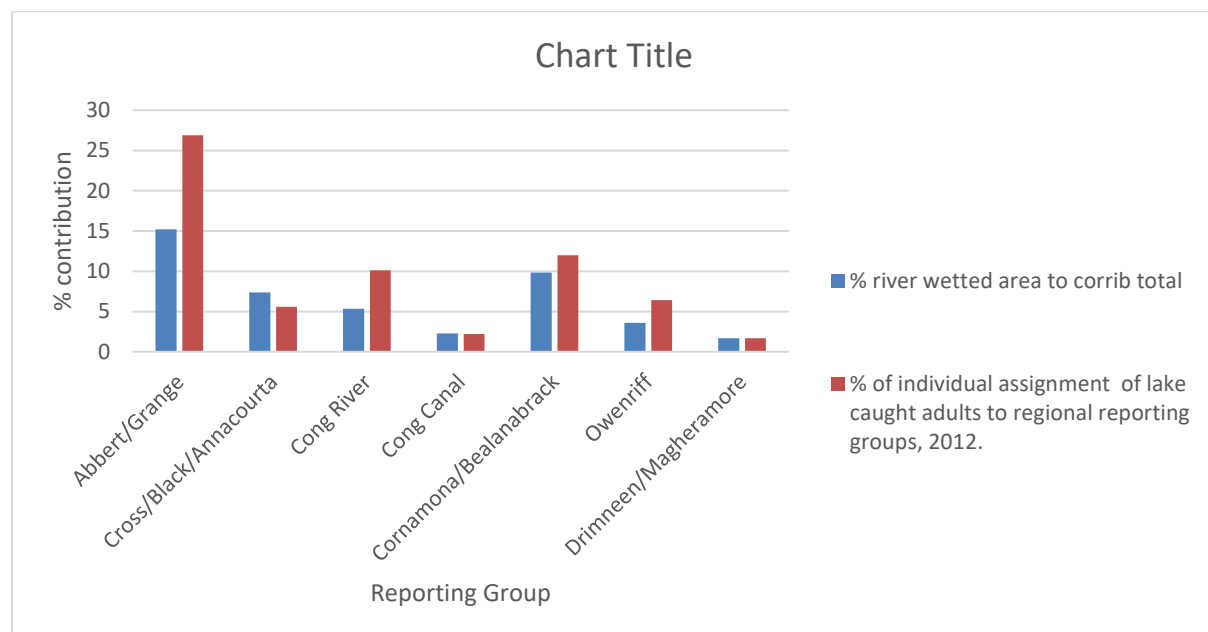
The results presented here clearly highlight the importance of the Clare River catchment to the overall production of brown trout to L. Corrib (Abbert and Grange rivers are tributaries of the Clare River system). The Clare system is the largest of all the L. Corrib sub-catchments and is also of significant importance in terms of Atlantic salmon production. While the Clare River is also known to support comparatively smaller proportion of river resident brown trout (i.e., trout that do not migrate to the lake at all but spend their whole life cycle within the Clare system from juvenile to adult), it is evident, from this study, that this catchment is particularly important in terms of salmonid spawning, nursery and adult waters.

In many cases there was a close geographical association between lake and river baseline samples, with a high occurrence of lake adult fish found within a 5 / 10km radius of their assigned river. In all instances, however, at least some adult fish, from each tributary, did show more extensive movement across the lake. It should be noted that the distribution pattern of lake adults reported here represents a spring lake sample, and that seasonal distribution patterns can vary depending on lake water temperature, feeding, spawning etc. Furthermore, previous studies investigating the movement of tagged Corrib adult lake fish (Toner, 1958 & Walsh and McCarthy, 1985) have suggested extensive movement of trout, from their various rivers, right across the lake. These reports are not in disagreement with the findings of this study. Thus, while adult brown trout assigning to the Abbert/Clare regional group tend to be found in the southern regions of the lake some individuals were also noted in the upper basin and adult trout assigning to the Cornamona/Bealanabrack appear to be more evenly distributed in the lake. Further studies incorporating seasonal sampling may help to elucidate the movement patterns of L. Corrib adult brown trout.

An interesting, but not entirely surprising, point to note is that the relative contribution of the various populations and/or regional groups correlates positively with the wetted area of each of the sub-catchments (Table 8). Thus, populations and/or regional groups representing the larger sub-catchments contribute the most to the lake adult brown trout stock. While this has also been noted in the L. Sheelin (Delanty *et al.*, 2020) and L. Ree brown trout genetic based studies (*in prep.*), other similar studies have shown this not to be the case (L. Mask - Massa-Gallucci, 2010; L. Derg and Moy studies - *in prep.*). Results from these latter studies indicate that brown trout inhabiting the largest sub-catchments do not all migrate to the lake but, instead, a large proportion spend their whole life cycle migrating from spawning to feeding grounds within the river (fluvial-adfluvial life history, see Ferguson *et al.*, 2019).

Table 8. River wetted areas and % contribution of associated populations and/regional groups to the L. Corrib adult lake stock.

	wetted area (km ²)	% river wetted area to corrib total	% of individual assignment of lake caught adults to regional reporting groups, 2012.
Corrib River Network	5748.81		
Abbert/Grange	873.64	15.2	26.9
Cross/Black/Annacourta	423.4	7.37	5.6
Cong River	306.79	5.34	10.1
Cong Canal	130.74	2.27	2.2
Cornamona/Bealanabrack	565.62	9.84	12
Owenriff	206.552977	3.59	6.4
Drimneen/Magheramore	97.629673	1.70	1.7



L. Corrib ferox trout

The results of this study relating to the Cong River deserve special consideration. The Cong River supports stocks of salmon (*Salmo salar*), Eurasian trout (*S. trutta*) and ferox trout (*S. ferox*), with this short river providing spawning and nurse grounds for all three salmonids. The association of ferox trout to the Cong River was not surprising, as ferox trout has been previously reported to occur and spawn in the lower reaches of this river (Gargan *et al.*, 2020). Thus, the results reported in this study

confirm these earlier reports. The comparatively large contribution (~10%) of Cong River ferox trout to the L. Corrib adult trout stock in 2012 is, however, intriguing. Given its small carrying capacity (i.e., Cong is relatively small river with limited spawning area), it seems unlikely that this river could support a sufficiently large ferox population to support this level of contribution to the lake adult stock. It is also important to note that in addition to ferox trout, the Cong River also supports a comparatively smaller brown trout population. Cong River samples used in this study, however, were targeted to areas where ferox trout are thought to spawn, hence, it is assumed that results presented here represent the contribution made by ferox.

To better understand this result, it is also important to emphasise that the proportion of the river populations and/or regional groups' contributions to the lake stock reported here (Table 6), do not take into consideration the age structure of lake adult fish. Thus, in each case (i.e., river and/or regional group contribution), the estimated contribution values represent the accumulated total from fish of all age groups. Ferox trout are long lived (up to 12yrs noted for L. Corrib) in comparison to other brown trout (4 to 5yrs on average). Thus, the ~10% contribution of the Cong River ferox (2012), in fact, is likely to represent some 6 to 8 age classes. Most ferox trout seem to migrate from the Cong River as 2 two year old fish and, for the most part, few ferox live past 10 years (IFI unpublished data).

Notwithstanding the points above, genetic data clearly supports the existence of large numbers of ferox trout in L. Corrib. Since ferox trout can only be reliably identified on the basis of their size, colour and age, prior to this genetic based study there was no robust approach to effectively assess their number in the lake. Given that ferox trout must grow to be large and old, it is feasible that many of the smaller adult trout, caught during the lake surveys, are in fact ferox that could not be easily differentiated from non-ferox trout. An examination of the length and associated age structure of all lake caught adults in the 2012 survey (O'Grady & Delanty, 2013) provides important insights into the results presented in here.

Similar to all Eurasian trout, young ferox trout predominantly feeds on invertebrates. This feeding behaviour continues until they reach sufficiently large sizes to be physically able to consume fish prey (Keeley & Grant, 2001, Ferguson & Prodöhl, 2022). While the age/length of the switch in feeding behaviour varies among individuals, the rate of growth, from that point onwards, increases for ferox in comparison to non-ferox trout. Before this switching point, however, given similar diets, no differences are expected in age at length between ferox and non-ferox trout.

In Fig. 11, the estimated length at age for genetically assigned ferox (linked to the Cong River 2012) and non-ferox (linked to the remaining river and/or regional groups baselines 2012) are plotted for

comparison. The results of this analyses suggest a clear fit with the age at length model outlined above. Thus, while ferox and non-ferox trout cannot be distinguished at early ages, there is clear evidence for a size switch around 4yrs+, when a bias towards larger sizes is obvious for genetically assigned ferox trout. These results not only support the genetic identification of ferox trout in L. Corrib, but also suggest that the diet switch to piscivory occurs somewhere in the mid 40cm length range.

Further compelling evidence in support of the results presented in this study is provided by the examination of the distribution of these presumably smaller ferox trout in the lake. Thus, the distribution of most of the lake fish caught during the 2012 survey, assigning to the Cong (ferox) River baseline, is a very good match with the distribution of the known adult ferox fish from the Gargan *et al.*, 2020 study, based on radio-tracking as shown in Fig. 9. The noted discrepancies (i.e., the presence of ferox trout in the southern areas of L. Corrib) are explained by the fact that the Gargan *et al.*, 2020 study was limited to the northern regions of L. Corrib. The genetic analyses of the known ferox trout used in the Gargan *et al.* (2020) radio-tracking based study, provides unambiguous validation of genetic typing for ferox trout. On the basis of genetic assignment, it was possible to identify all adult trout caught in 1996 and 1997, and in the 2012 survey as “ferox” and “non-ferox” trout. The fact that most of the identified ferox trout were actually large specimens (>48 cm in length), corroborates the conclusions presented in here. In summary, it is likely that the proportion of ferox trout among the L. Corrib adult trout have been somewhat underestimated prior to this study.

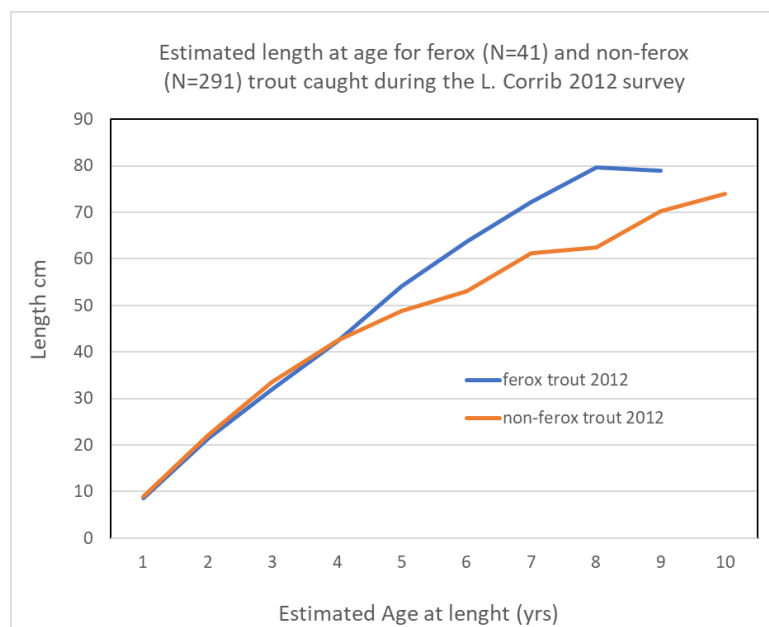


Figure 11. Estimated length at age for genetically assigned ferox and non-ferox trout caught in the 2012 lake survey (O’Grady & Delanty, 2013).

While the multiple lines of evidence supports the presence of large number of ferox trout in L. Corrib, it is still difficult to reconcile these large numbers with the Cong River, given its limited carrying capacity as outlined earlier. One possibility is that there are additional ferox trout spawning locations, which were not covered in the current, or the previous study of Massa-Galluci *et al.* (2010). While this hypothesis cannot not be dismissed without additional survey, it is interesting to note that the assignment probabilities (P) of lake adult to Cong River was, on average, larger than 0.8 indicating a very strong genetic affinity with Cong River ferox trout. An alternative and perhaps more likely explanation is that the L. Corrib ferox trout consist of a metapopulation comprising a series of interconnected spawning groups that include the Cong River, in addition to other spawning aggregations localised in suitable areas in the lake, possibly close to the Cong River. From observations it has been noted that ferox trout spawn within a very limited section of the lower reaches of river (close to L. Corrib), and on average an estimate of somewhere between 50 to 80 pairs of ferox fish may be using this area for spawning annually (*pers comm.* M. Varley & P. Gargan). Given the limited carrying capacity (spawning grounds), it is likely that ferox juveniles quickly migrate to the lake for feeding and growth. There is increasing evidence in the literature describing that lake spawning behaviour in salmonids, including the Eurasian trout (Kennedy *et al.*, 2021; Ferguson *et al.*, 2019) is more common than originally thought. The large number of islands in L. Corrib with suitable shallow shores, potentially provide ideal spawning grounds for the large ferox trout. Indeed, it has been suggested by some local anglers that ferox trout may be spawning around some of the islands within the northern section of the upper Corrib basin. It is worth noting that small salmonid fish have been previously observed in the shores of some of the islands in L. Mask, and when genetically typed were found to be trout (P Gargan, *pers comm.*). Reports from some L. Corrib anglers suggests that similar shoreline spawning may be occurring around some of the upper basin island of Corrib. Additional surveys targeting small fish in these areas in L. Corrib would be required to test this hypothesis.

An obvious implication linked to the occurrence of a large number of ferox trout in L. Corrib is that young ferox (less than 4yrs) have been particularly vulnerable to angling. In particular because they seem to concentrate in the northern regions of the lake, an area well known by active trout angling activities. Thus, while large ferox trout are invariably protected, young ferox trout are not. Additional studies would be required to evaluate the potential impact of angling of younger (<4yrs) ferox trout in the overall abundance of ferox trout in L. Corrib.

Another point to note is ferox trout are known to be late maturing fish (Went, 1979). The low numbers of ferox observed to be spawning in the Cong River only representing these older fish. A large proportion of the ferox lake population would be under this age and so not likely to be contributing

to the ferox spawning effort annually. The percentage of ferox trout identified during the 2012 study that was under 45cm was 49%, with 63% of that study age 4+ or younger (Figure 12).

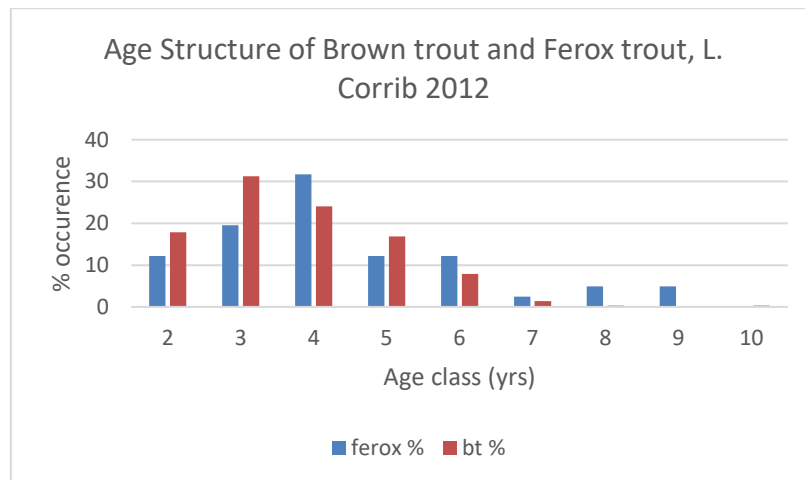


Figure 12. Age structure of ferox and brown trout recorded in the 2012 lake survey (O’Grady & Delanty, 2013).

In summary, this study provides compelling evidence indicating that the spawning of L. Corrib ferox trout is associated with the lower reaches of the Cong River and possibly other spawning areas in L. Corrib. The results presented also indicate that the Cong Canal is a spawning area for L. Mask ferox trout. Thus, both L. Corrib and L. Mask ferox trout were found to spawn in the Cong River. This is not surprising as fish from the Cong Canal can pass through the fish screen/grid in place on the Cong River (located near the top end of the Cong hatchery). Indeed it is possible, and has been reported from IFI hatchery staff, that adult brown trout returning from L. Corrib do migrate up through the fish screen/grid moving upstream to the canal and possibly onto L. Mask.

It is important to note, however, that despite a few exceptions, there appear to be only very limited exchange between ferox trout from L. Corrib and L. Mask (in both directions). Also of significance is that the direction of juvenile migration, a trait known to be under genetic control, is distinct for both populations (i.e. inflow migration for L. Corrib versus outflow migration for L. Mask). Since this study was focused on L. Corrib brown trout, it is not possible to confirm the existence of other ferox populations in L. Mask in addition to that of Cong Canal.

Examining the possible impact(s) of the extensive stocking history in the lake and inflowing tributaries on the wild L. Corrib brown trout populations

Similarly to what has been observed in other brown trout genetic based surveys in Ireland (Delanty *et al.*, 2020), there was no evidence indicating any impact(s) resulting from the known extensive stocking history from Roscrea farm fish in L. Corrib. As clearly shown here, Roscrea farm derived brown trout are genetically very distinct from wild brown trout and, as such, they can be easily identified in the wild. The fact that they were not found in this comprehensive survey, confirms what has been reported by Delanty *et al.* (2020) for L. Sheelin. Thus, when stocked as fry, juveniles or adults, Roscrea farm derived fish have extremely poor survival in comparison to the wild equivalent, so that any impact of stocking, particularly if these are not continuing, is negligible.

A brown trout hatchery, located towards the lower end of the Owenriff main channel in Oughterard, has been in operation for many decades. The main difference is that, currently, the Oughterard hatchery employs local wild brown trout, taken from the lower reaches of the Owenriff river as broodstock for the production of swim up fry to be stocked into the Owenriff (supplement stocking). Thus, in principle, no major genetic differences are expected between broodstock and local (Owenriff) wild fish.

While this is a potentially preferable approach for stocking (i.e. using local genetic material), the examination of the genetic profile of the Oughterard hatchery fish (broodstock fish and fertilised eggs) suggests that a mixture of fish, mainly from Cornamona, Bealanabrack, Owenriff, and Cong River, in addition to Owenriff have been used. Most likely this is associated with the choice of location(s) for capturing adult fish (i.e. lower reaches of the Owenriff river). There is increasing evidence from radio-tracking studies (of lake systems) that adult brown trout may enter rivers, other than their own natal river, for short periods before they leave to return to their natal river to spawn (Gargan *et al.*, 2020 and Kennedy *et al.*, 2021). It is, thus, very likely that some of these adult fish have been inadvertently caught and used as Owenriff broodstock for the Oughterard Hatchery, when they are in fact from other tributary rivers. Results from the individual assignment analyses, taking into consideration location of capture of adult fish, further corroborate this hypothesis. Thus, there is significant evidence from the genetic data that adult trout from Cornamona, Bealanabrack and Cong River can disperse to areas close to the lower reaches of the Owenriff River. For ferox trout (from the Cong River), this is further supported by the radio-tracking study of Gargan *et al.* (2020).

In summary, as confirmed from the genetic analyses, fertilized eggs from the Oughterard hatchery represent a combination of different genetic architectures resulting from a mixture of parental fish

from distinct locations. While it is possible that offspring resulting from the correct parental combination (i.e. Owenriff male versus Owenriff females) may survive in the wild once released, the fate of offspring from other parental combination is less certain. Current Oughterard hatchery practices involving the use of fish considered to be of Owenriff origin as “local” Owenriff broodstock with subsequent release of swim up fry into the Owenriff River catchment (supplemental stocking), has been ongoing since 2015 only. For some 110-120 years prior to that time, some 150,000 to 300,000 swim up fry, produced at the hatchery, were released throughout the L. Corrib system annually.

While it is difficult to assess the impact(s) of this stocking programme, it is unlikely that this has significantly affected the genetic integrity of L. Corrib brown trout populations. The rationale for this conclusion is that, by design, this continuous stocking programme (spreading genes from the West/Northeast group) throughout the system, would, in principle, have promoted homogenisation of genetic variation between and among populations (i.e., through man-induced gene flow). Results from this study, however, clearly shows that this has not been the case. Thus, there are clear genetic differences between brown trout populations from the two major broader geographical groups (East and West/Northeast). Albeit to a lesser extent, significant genetic differences are also evident among populations within each of these major geographical groups.

The results presented here, however, do not entirely rule out at least some impact. The intriguing link between Cong and Owenriff, identified from the different analyses, may provide some evidence for this. Thus, since the Oughterard hatchery has been operating for over 100 years, it is possible that this link is not natural, but driven by the routine inclusion (man-mediated gene flow) of Cong River fish among the Owenriff broodstock. To further support this hypothesis, it is thought that exchanges of fish (broodstock and/or offspring) from the Oughterard hatchery to the Cong hatchery have happened on occasions. As the Cong hatchery is now only a salmon rearing facility this practice no longer occurs.

The impact the salmon rearing/ranching facility at Cong village has had (and possibly continues to have) on wild salmonid stocks within the Cong River is not clear. It is clear though that salmon now dominate the river (Gargan/IFI report 2020). All three salmonid groups (salmon, brown trout and ferox trout) compete for the same spawning and nursery grounds, which is limited in this river already, and it is unlikely that the Cong River has the ability to support healthy populations of all three species concurrently.

In conclusion, the impact(s) of any past stocking activities are not possible to fully determine at this time, confidently. However genetic results summarised above do suggest there is little evidence of

any substantial impact. Particularly due to the natural high mortality of swim up fry, which is the stage that fish are released from the Oughterard hatchery. Ferguson (2007) summarises several reasons for the often poor performance of stocking programmes including: number stocked relative to wild fish; reduced survival of hatchery produced fish in the wild due to the removal of mating choice; transport and stocking technique; natural environmental conditions; physiological differences; morphological differences; feeding and predator-avoidance behaviours; greater angling susceptibility; reduced breeding ability; assortative mating; reduced survival of hybrid offspring.

Future genetic programmes

The current study has highlighted that sample size from a small number of rivers was too low for accurate assignment and, hence, they were combined with a near neighbouring river. While this approach is sound when genetic differences are negligible, any future studies should consider securing adequate sample sizes to eliminate any possible bias resulting from sample merging. A comparatively large number of adult fish could not be assigned with confidence, using the $P > 0.7$ criteria to any of the currently identified populations and/or regional groups. This criterion may be considered too stringent but, it allows for good confidence in the results. While, it is acknowledged, that fish assigning with less likelihood (smaller P values) may still be assigning to the correct population and/or reporting groups, others, in the absence of the correct baseline (source population) may incorrectly assign to a genetically related population (i.e., the best next population match). This study, however, has shown that this type of error should not be of major consequence as individuals are still likely to assign to the correct broader geographical group. Nevertheless, to minimise the impact of this type of issue, it is also recommended that tributaries not previously sampled should be included in any future surveys and genetic studies. Such rivers would include tributaries from across the Clare River catchment, Fallomer (Owenwee), Cregg, Lough Kip, Derrylaura and Currarevagh systems.

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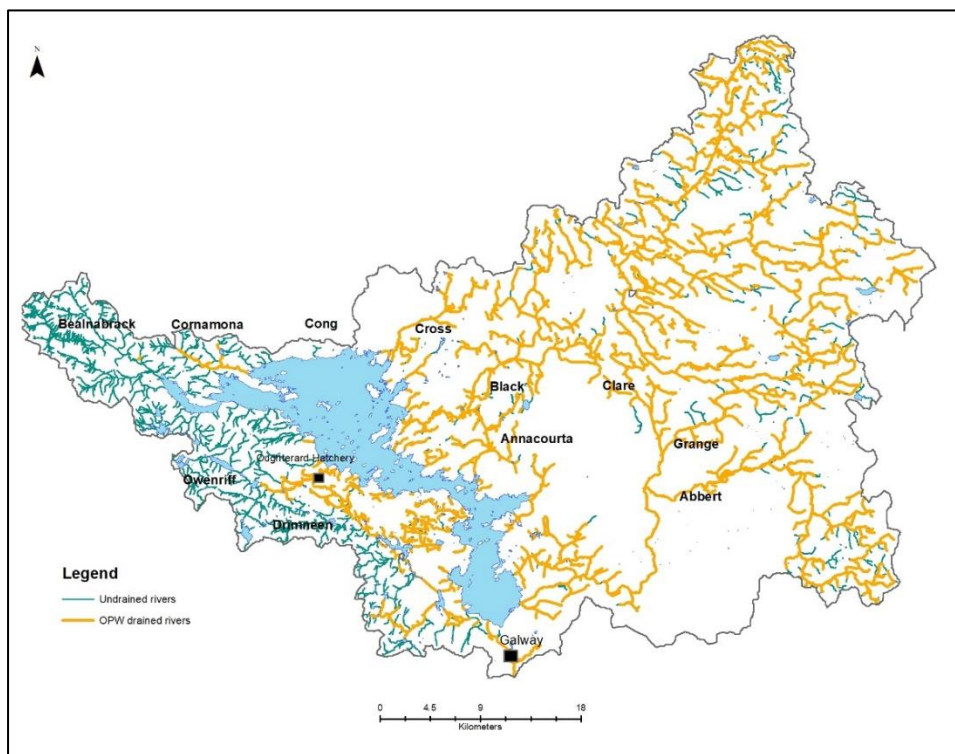
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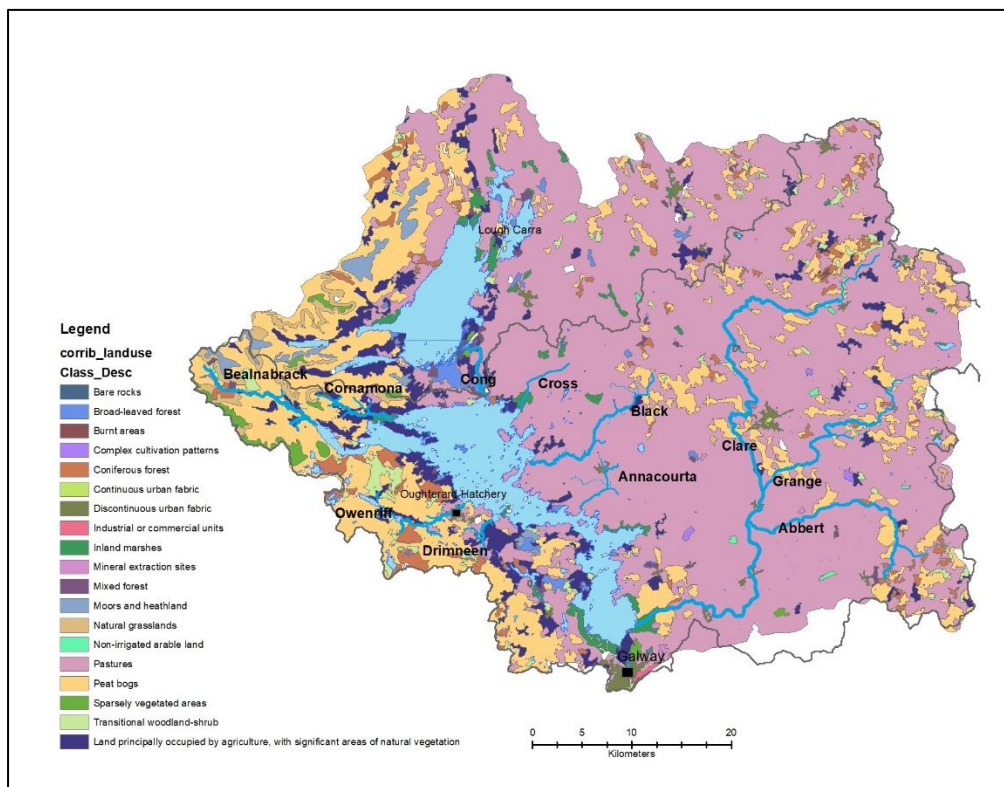
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APPENDIX A-G Supporting Maps

A - OPW drainage scheme across L. Corrib catchment (<https://www.floodinfo.ie/>)

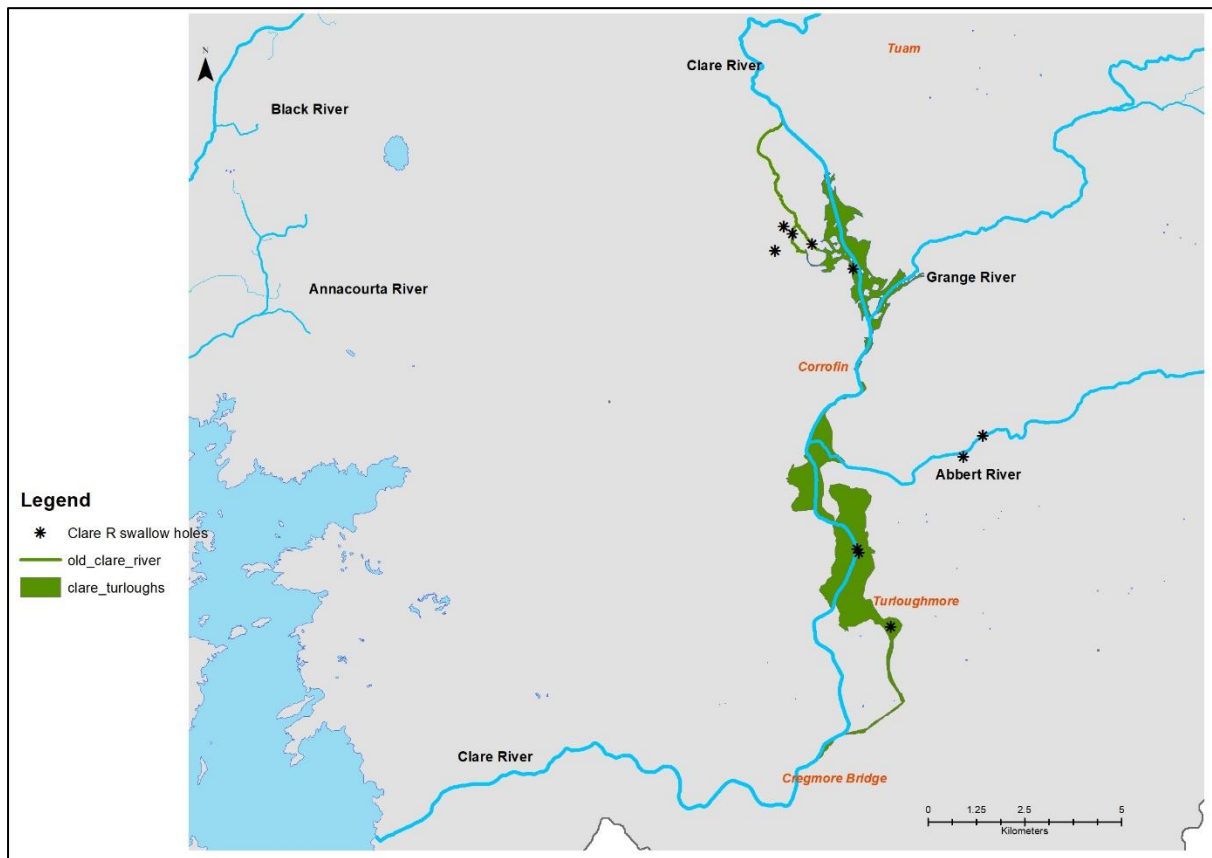


B – L. Corrib Corine - Landuse (<https://gis.epa.ie/EPAMAPS/>)



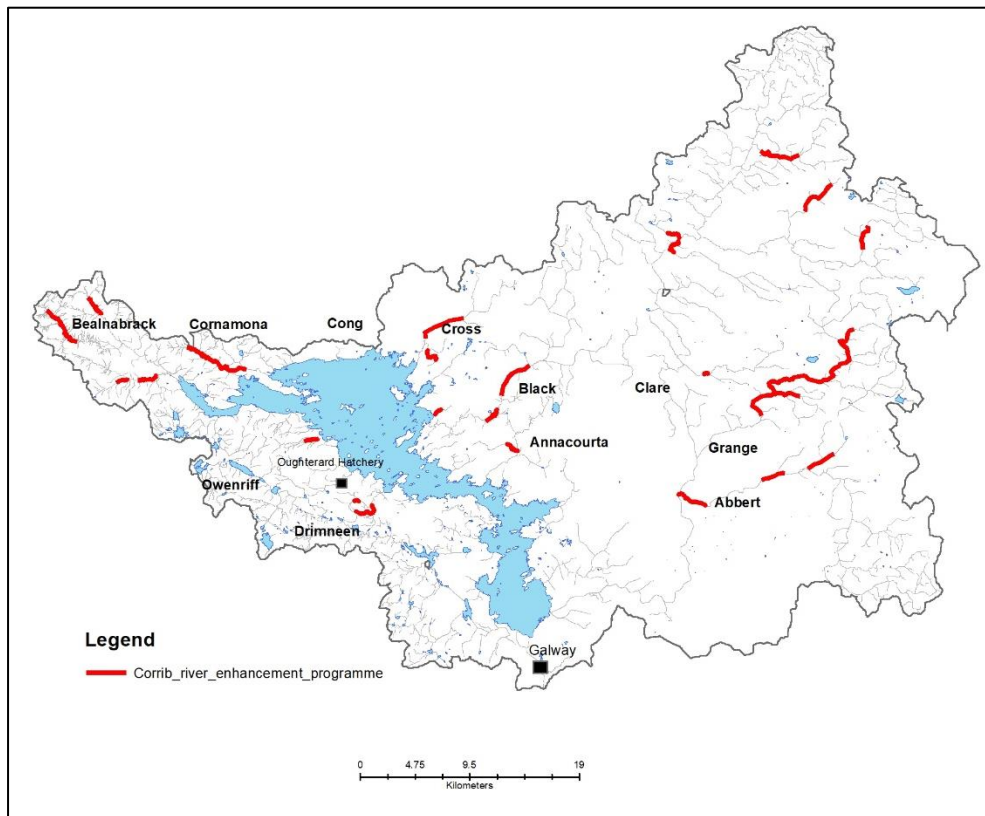
C - Clare River prior to 1850's drainage works (*approximate*)

(redrawn from OSI historical 6" maps - <https://webapps.geohive.ie/mapviewer/index.html#>)

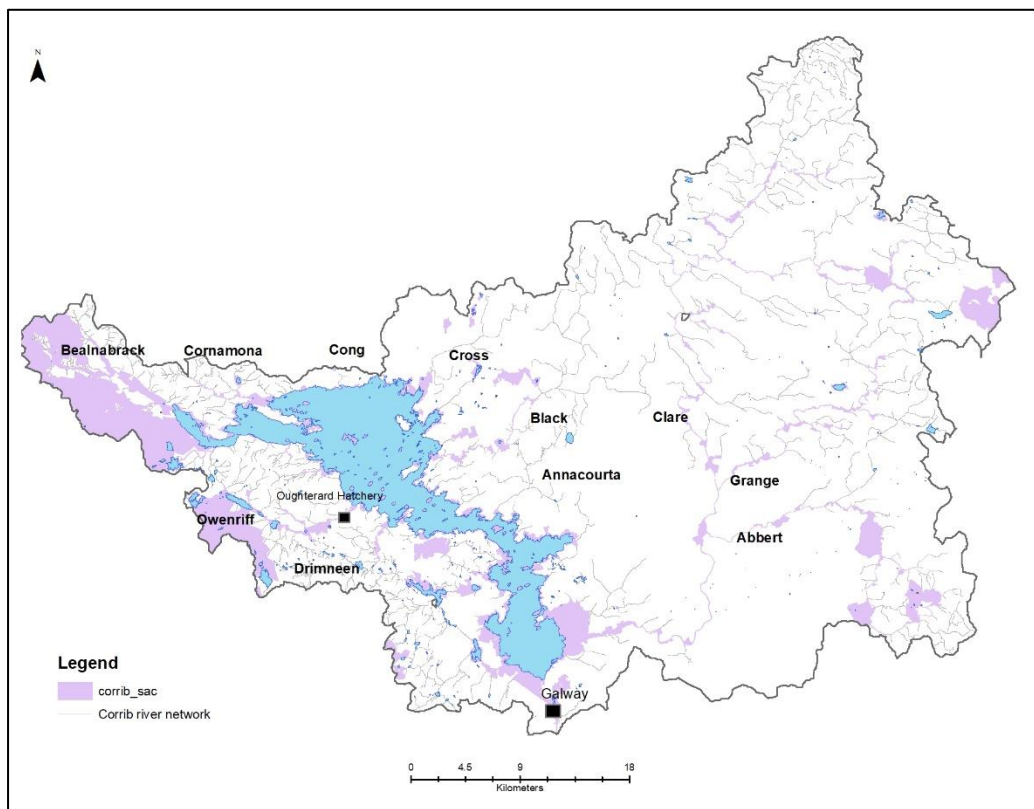


Flooding event Nov. 20th 2009 – Cloonkeen Lough is evident again

D – L. Corrib TAM & EREP Enhancement Works (source: IFI, 1998 – 2014)

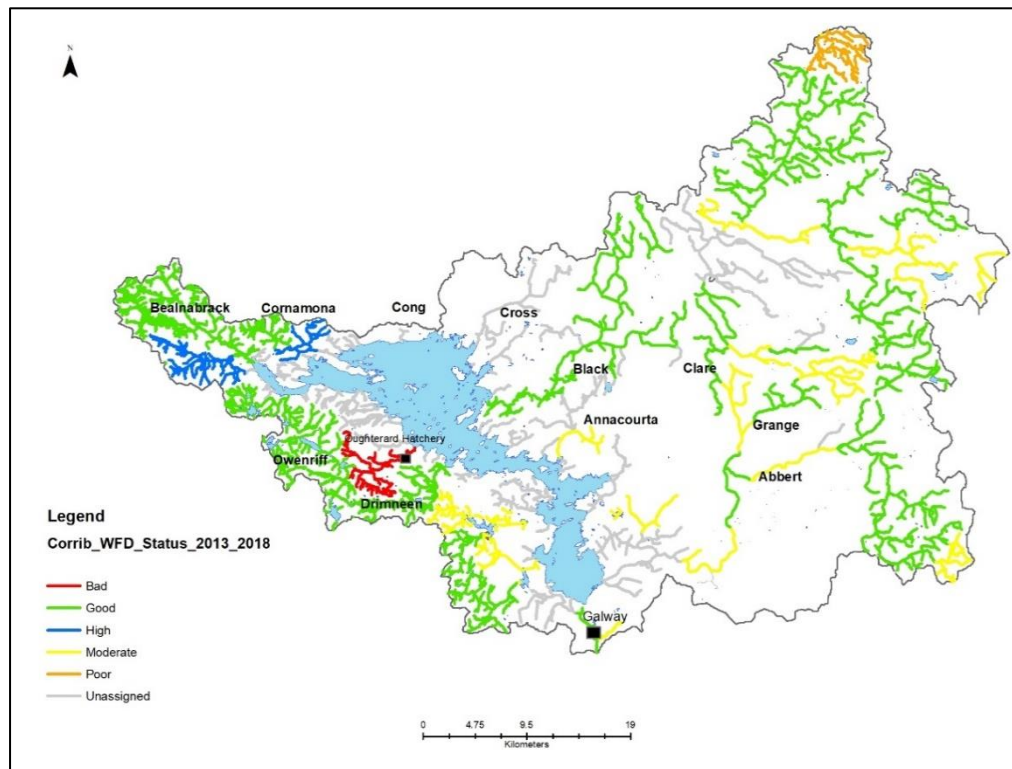


E – L. Corrib SAC (source: National Parks & Wildlife Service)

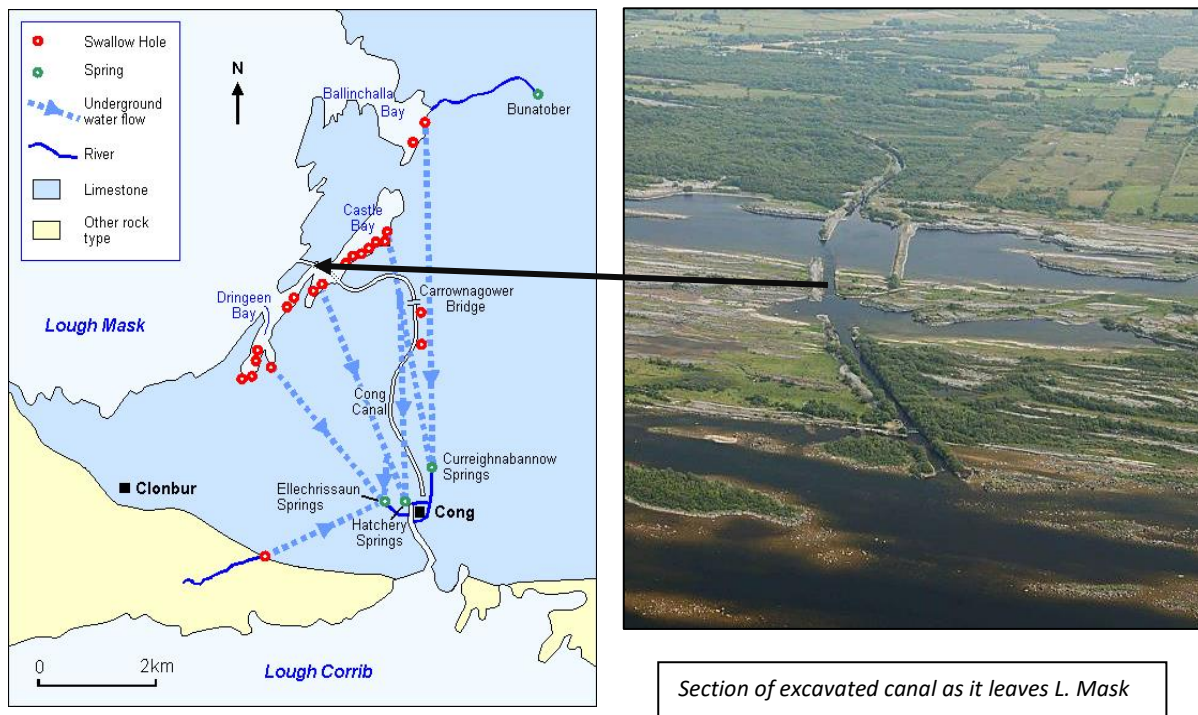


F - EPA – 2013-2018 WFD Status for the L. Corrib river network (source:

<https://gis.epa.ie/EPAMAPS/>)



G – L. Mask to L. Corrib underground water flow



(Redrawn from maps taken from GSI -

<https://dcenr.maps.arcgis.com/apps/webappviewer/index.html?id=7e8a202301594687ab14629a10b748ef>)

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A large, dark blue curved graphic element that starts from the left edge and curves upwards and to the right, filling the bottom half of the page. It has a subtle gradient and a thin white line along its top edge.