

# Population Structure and Genetic Stock Identification of the Moy Catchment Brown Trout

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# **POPULATION STRUCTURE AND GENETIC STOCK IDENTIFICATION OF THE MOY CATCHMENT BROWN TROUT**

Beaufort Fish Genetics Group,  
School of Biological Sciences, Queen's University Belfast  
and  
Inland Fisheries Ireland



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Contributors to this report were as follows:

1) from Queen's University Belfast (QUB) - Beaufort Post-Doctoral Fellow Dr Caroline Bradley (laboratory, data analyses and report editing), Dr Rosaleen Hynes (laboratory and reporting editing) and Prof. Paulo Prodöhl (planning, data analysis, biological interpretation of results, report drafting and editing) and

2) from Inland Fisheries Ireland (IFI) – Dr Karen Delanty and Prof. Martin O'Grady (planning, sampling acquisition, biological interpretation of results, report drafting and editing).

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<sup>1</sup>Institute for Global Food Security, School of Biological Sciences, Medical Biology Centre, Queen's University, Belfast, Northern Ireland.

<sup>2</sup>Inland Fisheries Ireland, Citywest Business Campus, Co Dublin, Ireland

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## 1. Introduction

Changes in the physical and natural environment can significantly impact the long-term sustainability of *Salmo trutta* populations, a species within the Eurasian (*Salmo*) trout species complex (Jonsson & Jonsson, 2009; Smialek *et al.*, 2021; Ferguson & Prodöhl, 2022). Over the last century, urbanization and agricultural activities leading to the loss and fragmentation of natural spawning and nursery habitats, and the introduction of non-native species among other factors, have resulted in fluctuations in brown trout productivity and raised concerns about the health and sustainability of brown trout populations in the Loughs Conn & Cullin and River Moy catchment areas.

Preserving genetic diversity, particularly adaptive variation, is crucial in the management and conservation of biological resources. Adaptive genetic variation allows populations to cope with local environmental changes, ensuring their long-term survival. Habitat degradation and other related human activities, however, pose a significant threat to the maintenance of adaptive genetic variation within and among populations. Therefore, regular genetic monitoring of populations is essential in developing effective management and conservation plans (Hoban *et al.*, 2022).

Given this background, Inland Fisheries Ireland (IFI) commissioned QUB (Beaufort Fish Population Genetics Research Group, Queen's University Belfast) to carry out a genetic study of the Moy Catchment brown trout with the aim of generating a baseline for future studies in addition to investigate the potential changes in the genetic composition of populations due to the changing environment. The study is based on a large-scale biological survey data collected in the Moy Catchment from 2011 to 2016. The findings from this extensive research were presented in 2017 at the International Brown Trout Genetics Conference, organised by IFI (<https://www.fisheriesireland.ie/publications?f%5B0%5D=categories%3A303>). The outcomes and insights from this study are included in this report. It is part of an ongoing series of brown trout population genetic reports published by the IFI following the conference.

The project received monetary support from Lough Conn & Cullin Anglers Association. This angling club also contributed adult trout scale samples to the study. Adult sea trout scale samples were provided by private charter boats operating in the Moy Estuary, specialising in sea trout fishing.

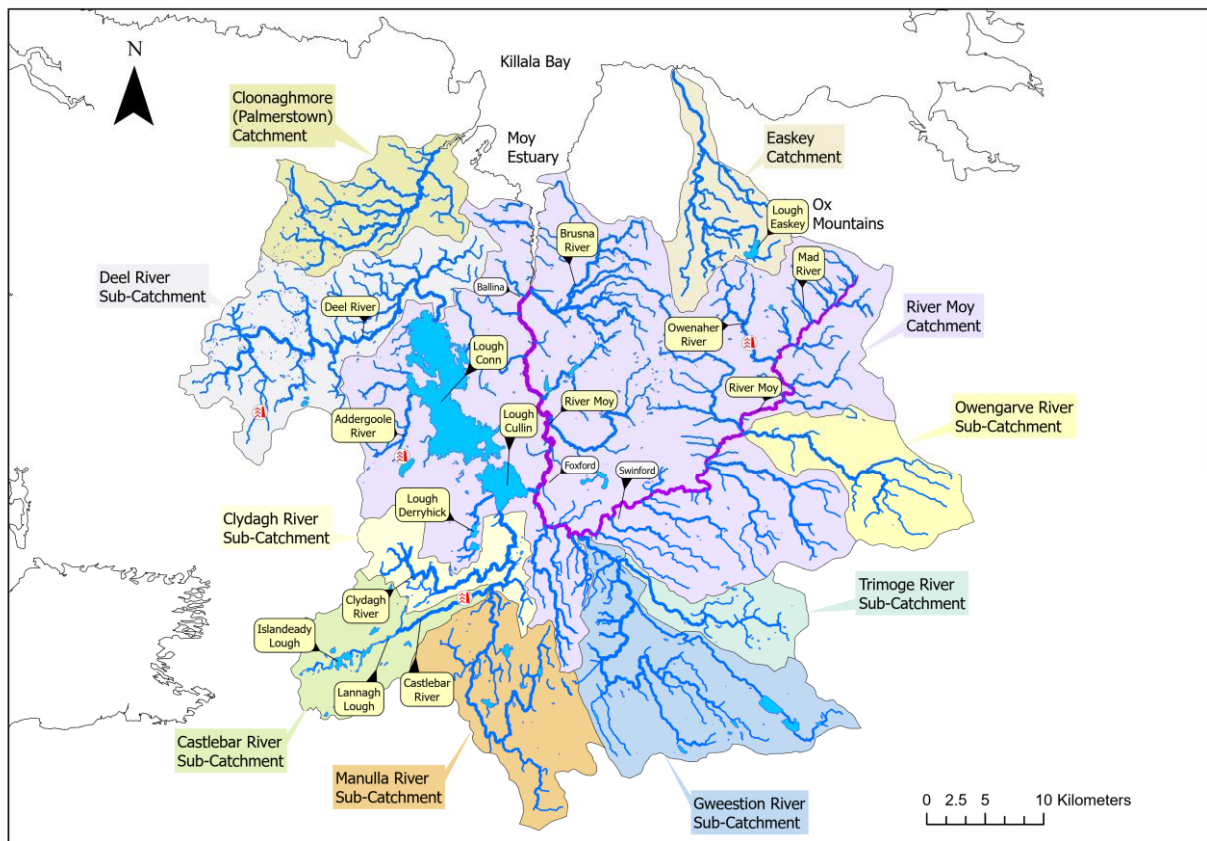
The results of the population genetic study of Eurasian trout from the Moy Catchment system are reported here.

## 2. Catchment Study Area

The area of study, illustrated in Figure 1, comprises primarily the large River Moy catchment, but also includes the Easkey, and Cloonaghmore catchments (referred to as Palmerstown hereafter). These two catchments are close to the Moy catchment, either flowing into the Moy Estuary (Palmerstown) or easily accessible to it (Easkey). Their inclusion in this study was important to account for potential geneflow mediated by anadromous *S. trutta* (sea trout).

### 2.1 Moy Catchment

The Moy catchment drains a vast area of approximately 2108 km<sup>2</sup>, with an extensive river network system and several lakes most notably Loughs Conn and Cullin (Fig. 1). The River Moy rises at the foot of the Ox Mountains in Co. Sligo and flows for 110 kms. For the greater part of its length it flows southwestward, entering Co. Mayo and passing near Swinford before turning north and heading for the town of Ballina, where it enters the sea at Killala Bay, via the long Moy estuary (8 km). The broader River Moy system is such a large catchment that it is linked to several substantial sub-catchments, most notably the Deel, Castlebar, Manulla, Gweestion, Trimoge and Owengarve Rivers, along with a host of smaller ones (Fig. 1).



**Figure 1.** Area of study including main sub-catchments comprising the broader River Moy (highlighted in deep purple colour) catchment system. Symbols in red on the map indicate the presence of natural waterfalls and barriers.

The River Moy catchment area is characterised by a combination of geological features, primarily consisting of calcareous rocks, but also incorporating a band of siliceous rocks that extends midway across the catchment (Appendix I, GSI, 2023). Pastures are the dominant type of land usage, but the

area also contains a significant coverage of peat bog (Appendix II, CORINE, 2018). Several significant Special Areas of Conservation (SAC) are contained within the Moy Catchment, including the entirety of the River Moy, Moy Estuary, and Killala Bay, as well as the Bellacorick Bog Complex and the Ox Mountain Bogs. These SACs are acknowledged as areas of significant importance for a range of habitat types, flora, and fauna (NPWS, 2023). Both Lough Conn and Cullin are listed as Special Protection Areas (SPA) (NPWS, 2023).

Water quality across the catchment is variable. The most recent WFD status EPA report for the Moy catchment is presented in Appendix III. The Moy catchment has suffered from water quality challenges historically, and the impacts of these issues persist today. At present, the Castlebar and Tubbercurry Rivers' sub-catchments are considered at risk due to declining water quality (<https://gis.epa.ie/EPAMaps/Water>). Although there have been improvements in wastewater treatment (WWT) plants in various parts of the catchment, water quality remains a significant concern in specific sections, especially the Castlebar River. Agricultural activities and river hydromorphology are major contributing factors to the pressures linked to the 'At Risk' areas of the Moy catchment (<https://gis.epa.ie/EPAMaps/Water>).

WFD Ecological Status and Fish Status for both Loughs Conn and Cullin are presented in Tables 1 and 2. In 2022, Fish Status for L. Cullin improved to Good, the first time since WFD surveys on the lake began (Corcoran *et al.*, 2023). Data from the Inland Fisheries Ireland (IFI) WFD fish monitoring programme indicates that the majority of monitored sites display good Riverine Fish Ecological Status, with only a small number categorized as Moderate (Kelly *et al.*, 2016, & 2017a & c). EPA River Waterbody WFD Status, for the reporting period 2016 – 2021, is presented in Appendix III.

**Table 1.** EPA WFD Ecological Status, L. Conn and L. Cullin

Reporting Period	L. Conn	L. Cullin
2010-2015	Good	Moderate
2013-2018	Moderate	Moderate
2016-2021	Good	Moderate

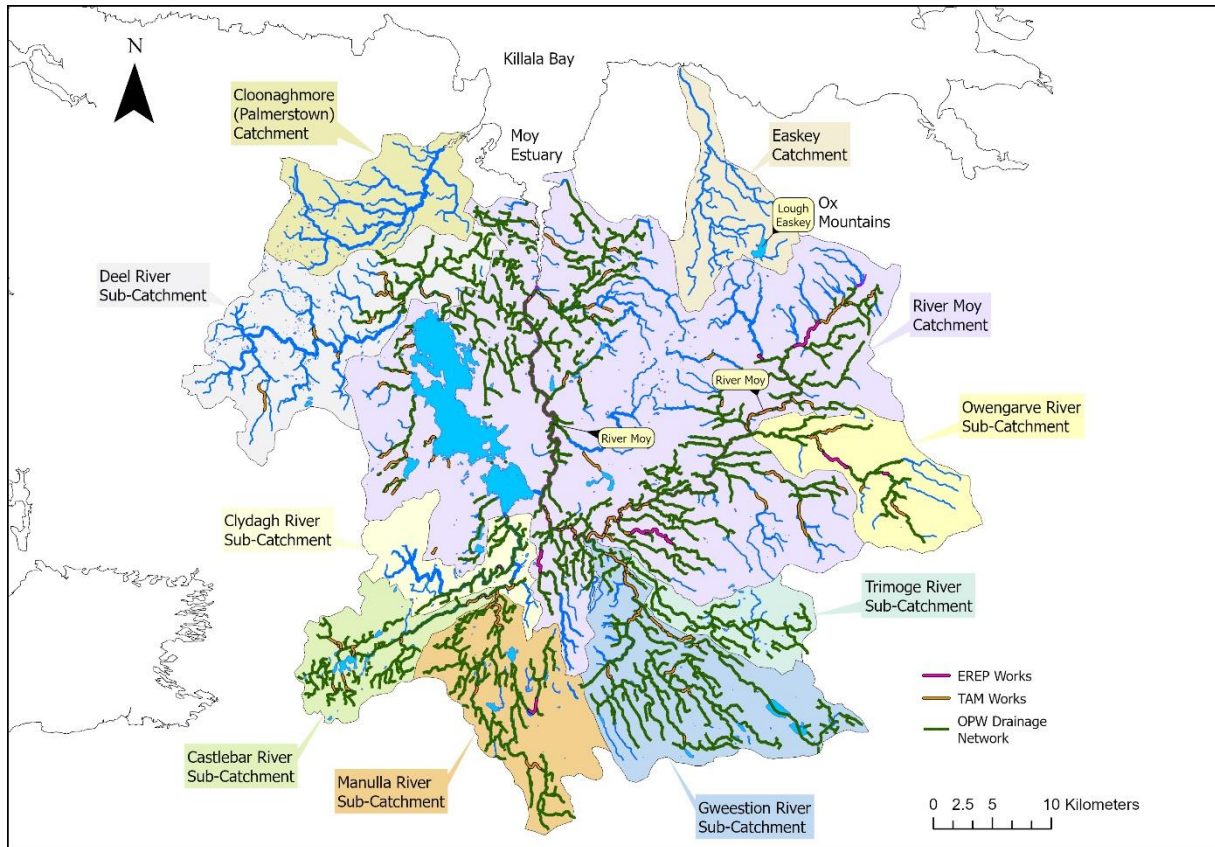
**Table 2.** IFI WFD Fish Ecological Status, L. Conn and L. Cullin

Reporting Year	L. Conn	L. Cullin
2009		Poor
2012		Poor
2015		Moderate
2016	Good	
2018		Moderate
2022	Good	Good

In the 1960s, the Office of Public Works (OPW) implemented a large-scale arterial drainage programme throughout almost the entire Moy catchment area (Fig. 2). This initiative aimed to enhance land drainage and agricultural productivity within the catchment. The programme, however, had a major impact on the catchment's hydrology, ecology, and fisheries. In particular, the drainage programme led to the alteration of the natural river through the widening, straightening, lowering of



the river bed, the loss of several lakes in the southern part of the catchment and/or the reduction in size of others, including Loughs Conn and Cullin (McGarrigle *et al.*, 1998). The programme also resulted in the removal of instream features, such as pools and spawning gravels, natural channel sinuosity, and both instream and bank vegetation, including riparian cover (O’Grady *et al.*, 2017).



**Figure 2.** Moy OPW drainage network, with TAM and EREP development sections identified.

Since the mid-1990s, the rivers and streams within the Moy catchment have been the focus of rehabilitation and enhancement works through two primary programmes: the Tourism Angling Measure (TAM 1994 -1999) and the Environmental River Enhancement Programme (EREP 2008-2015) (Fig. 2). These programmes have implemented instream and riparian measures to restore damaged or impacted sections of many rivers and streams, covering a total of approximately 117 kilometres (TAM approx. 100km and EREP approx. 17km) of channel. These measures include the introduction of spawning gravels, the development of pools and riffle areas, increased channel sinuosity, and the restriction of cattle access to the rivers (CFB 1997, O’Grady *et al.*, 1997a & b, O’Grady *et al.*, 1998, O’Grady & Delanty 2001, O’Grady & O’Leary 2007). Previous studies undertaken by IFI have shown that the drainage works have had a significant impact on the geomorphology and structure of the rivers, resulting in a reduction of suitable spawning and nursery habitats for salmonids and, thus, negatively affecting their productivity (O’Grady and Curtin, 1993; Gargan *et al.*, 2002; O’Grady, 2006).

Several fish species are present in the rivers within the River Moy catchment, including brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), sea trout (*Salmo trutta*), pike (*Esox lucius*), perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), European eels (*Anguilla anguilla*), 3 spined stickleback (*Gasterosteus aculeatus*), minnow (*Phoxinus phoxinus*), stone loach (*Barbatula barbatula*), lamprey (*Lampetra* spp) and flounder (*Platichthys flesus*) (O’Grady *et al.*, 1994, Kelly *et al.*, 2017c).



The Moy catchment is a designated salmon and brown trout fishery. The Moy main channel is renowned for being one of the most productive salmon rivers in the country (O'Reilly, 2007). Brown trout fishing is more popular in the Moy's larger tributaries and in Lough Conn (O'Grady & Delanty, 2001). The Moy Estuary is known as an excellent location for sea trout (anadromous *Salmo trutta*).

Natural falls and barriers were historically present on several rivers and streams around the catchment that made fish migration difficult or impossible. The more noteworthy of these structures are/were the falls on the Clydagh and Deel Rivers but also in the Castlebar, Mad and Owenaher Rivers (Fig. 1). A number of these falls and barriers were modified (opened up and made more passable), to alleviate fish passage, during the TAM river enhancement programme of works over the period 1995 to 1999. However, only those structures with significant upstream salmonid spawning and nursery waters above them were included in the programme of works. No works were carried out on the Mad or Owenaher Rivers and these natural impassable falls are still present.

Historical additional stocking within the Moy catchment area involved the introduction of farmed brown trout from the Roscrea fish farm. Such stockings were limited mainly to a small number of angling lakes located in the Castlebar region. These lakes included Loughs Lannagh, Bilberry, and Islandeady, which are collectively referred to as the Castlebar lakes. From the mid-1960s until 2021, these lakes were regularly stocked with farmed reared adult brown trout. On occasion, trout fry, summerlings, and yearlings were also released into the lakes and their inflowing systems (IFT annual reports 1957 - 1987, IFI unpublished data 2000 - 2021). Records indicated that over 1.2 million fish of farm origin of all life stages ranging from 0+ to 2+ year old adults have been introduced into the Castlebar lakes.

### 2.1.1 Lough Conn

Lough Conn has a surface area of 4,704 hectares (47,04 km<sup>2</sup>) and a maximum depth of 37.9m. The lake falls under typology class 12, a classification designated by the EPA for the Water Framework Directive. This classification indicates that the lake is deep, with a mean depth greater than 4 meters, has a surface area over 50 hectares (0.5 km<sup>2</sup>), and has high alkalinity exceeding 100 mg/l CaCO<sub>3</sub>. Lough Conn is linked to its adjacent lake, Lough Cullin via a narrow channel (see Fig. 1 and below for further details). The River Deel feeds into Lough Conn (north of the lake) and eventually flows out of Lough Cullin at its southern end, close to Foxford, before joining the River Moy main channel.

Lough Conn supports populations of trout, pike and roach, as well as salmon, perch, rudd (*Scardinius erythrophthalmus*), tench (*Tinca tinca*), eels and occasionally sea trout. Arctic char (*Salvelinus alpinus*), once present in the lake, are now extinct (Igoe *et al.*, 2001, O'Grady & Delanty, 2001. Kelly *et al.*, 2017b). While roach were first recorded in L. Conn in 2001, they were initially recorded in Derryhick lake, which flows into Lough Cullin, in 1996. This coarse fish species was probably introduced into Lough Conn in the late 1990's and into Derryhick lake during the late 1980's (O'Grady & Delanty, 2001). Another invasive freshwater species introduced into Lough Conn was zebra mussel (*Dreissena polymorpha*), which were first recorded in the lake in 2006 (Irish Times, 2006).

Lough Conn has a long-standing reputation as a fine brown trout and salmon fishery in the western regions of Ireland, dating back to the very beginning of angling in the area (O' Reilly, 2005). The main run of spring salmon enters Lough Conn from the end of March and continues right through to April.

The grilse run begins in May and continues into July (<https://fishinginireland.info/salmon/northwest/conn>).

### 2.1.2 Lough Cullin

Lough Cullin is a large, shallow lake situated towards the west of Foxford. It is connected to Lough Conn by a narrow inlet at Pontoon in Co. Mayo. The lake discharges its outflow directly into the River Moy, which is situated south-west of Foxford (NPWS). Lough Cullin has a surface area of 1019.3 hectares (10.19 km<sup>2</sup>) and a maximum depth of approximately 3m (O' Reilly 2007). The underlying geology of the lake consists mainly of granite with some areas of limestone present in the southern part of the catchment (NPWS). The lake is categorised under typology class 10, which means that it is shallow (less than 4m), has a surface area greater than 50 hectares (0.5 km<sup>2</sup>) and high alkalinity (more than 100mg/L CaCO<sub>3</sub>), as designated by the EPA for the Water Framework Directive).

Lough Cullin was once regarded as one of Ireland's premier brown trout fisheries, although it was often considered to be overshadowed by Lough Conn. From an angling perspective, Lough Cullin was renowned for its abundant stock of small-sized brown trout, weighing less than 0.5kg (O' Grady & Delanty 2001). The lake was also highly valued as a very important salmon fishery, with salmon runs occurring during the spring and summer months (NPWS, O' Reilly 2007). It is worth noting that all salmon destined for Lough Conn and its inflowing tributary rivers must pass through Lough Cullin. Lake surveys, carried out on L. Cullin, have reported the capture of roach, perch, brown trout, tench, rudd, pike, roach x rudd hybrids, salmon, and eels (O'Grady & Delanty 2001, Connor *et al.* 2019).

### 2.1.3 River Moy

The middle to lower reaches of the Moy main river channel, downstream from Foxford, do not support a significant adult brown trout stock, thus there is no recognised brown trout angling fishery in this section of the river (O'Grady *et al.*, 1994). This lower reach of the Moy main channel, from Foxford to Ballina, is a deep slow flowing river, which does not support a resident adult trout stock and has minimal spawning or nursery potential for salmonids.

However, many of the Moy sub-catchments, which discharge to the main stem upstream of Foxford, support substantial stocks of 0+ and 1+ trout, along with moderate to good stocks of adult brown trout (O'Grady *et al.*, 1994). Some of the larger Moy sub-catchments, which drain limestone areas, can support resident adult brown trout populations and may, thus, also contribute to the substantial adult stocks in Loughs Conn and Cullin. Examples of such river sub-catchments include Deel, Manulla, Castlebar, Trimoge and Gweestion (Fig. 1).

### 2.1.4 Moy Estuary

The Moy Estuary is eight kilometres (5 miles) long beginning at Ballina and running into Killala Bay. It is narrow and lined with stone walls for about three and a half miles before opening out into a huge basin. It then narrows again between Bartra Island and Enniscrone beach. Sea trout (anadromous *Salmo trutta*) congregate in the area to feed on the vast stocks of sandeel, sprat and shrimp (see [https://fishinginireland.info/salmon/northwest/river\\_moy\\_system/moy\\_estuary/](https://fishinginireland.info/salmon/northwest/river_moy_system/moy_estuary/)).

### 2.1.5 Palmerstown Catchment

The Palmerstown catchment (Fig. 1) drains an area of approximately 130km<sup>2</sup>. It was renowned for its sea trout stock, which is now largely depleted and struggling to survive (O'Reilly 2007). The catchment discharges into the northwest region of Killala Bay.

EPA River Waterbody Status for this catchment, over the three reporting periods (2010-2015, 2013-2018 and 2016-2021), indicates that the overall assignment and ecological status of the catchment has been Good (EPA, 2023). The underlying geology of the area consists mostly of limestone, with some sandstone and shales (GSI, 2023). The primary land use within the catchment is pasture, with some peat bog located in the upper regions. The upper reaches of the catchment fall within the Bellacorick Bog Complex SAC (NPWS).

The Palmerstown catchment was included within the broader River Moy system study because it is a neighbouring sea trout catchment that discharges into the same bay as the Moy. Therefore, there is potential for sea trout from either system to stray and or mix.

### 2.1.6 Easkey Catchment

The Easkey catchment drains an area of 101km<sup>2</sup> and flows into the Atlantic Ocean through a short estuary located along the coast between Killala Bay and Sligo Bay. This spate river stretches approximately 20km in length, originating from Lough Easkey located high in the Ox Mountains (Fig. 1). While the Easkey River is primarily known as a salmon river, it is also associated with a significant run of sea trout in July and August (O'Reilly, 2005). The river flows through mountain bog and rough pasture before emptying into the ocean downstream of Easkey village.

EPA River Waterbody Status for this catchment, over the three reporting periods (2010-2015, 2013-2018 and 2016-2021) indicates that the overall assignment and ecological status of the catchment has been Good (EPA, 2023). However, the Buncrowley sub-catchment has been assigned a Moderate status over the same report periods. The geology underlying the lower reaches of the catchment is limestone, while the upper reaches comprises schist and gneiss (GSI, 2023).

Differently from the Moy and Palmerstown Rivers, which flow into Killala Bay, the Easkey River discharges into its own estuary, a relatively small area that serves as potential feeding grounds for sea trout. However, small sea trout migrating from the Easkey River could potentially travel to nearby Killala Bay for feeding.

## **2.2 Study Objectives**

The main objectives of the project were:

- To establish a genetic baseline and to describe the contemporary brown trout population genetic structure of the River Moy and its main sub-tributaries.
- To quantify the relative contribution of identified populations to the adult brown trout fishery to Loughs Conn and Cullin (Genetic Stock Identification).
- To assess the potential impact(s) of the stocking history involving farmed derived fish on the wild brown trout populations inhabiting the broader River Moy catchment.
- To assess the impact of barriers (natural and manmade) to fish migration and their potential impact(s) on contemporary population genetic structure.

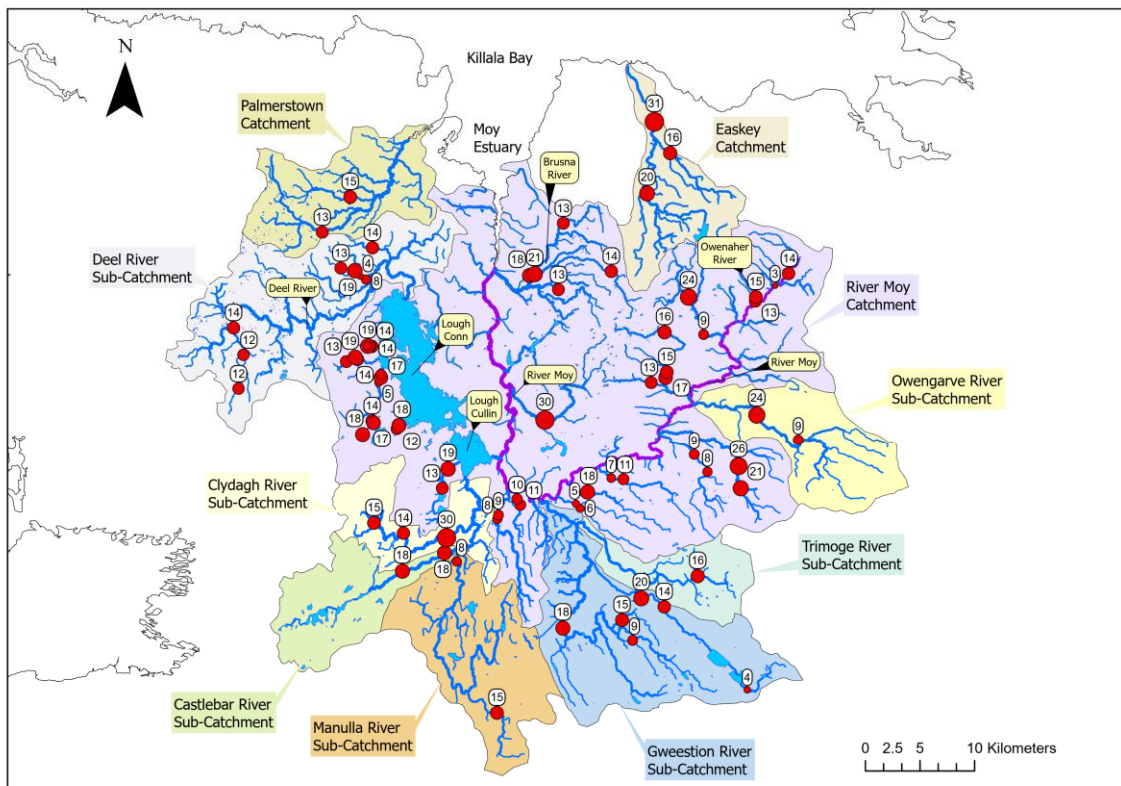
- To identify the potential source populations within the broader River Moy catchment and adjacent catchments, as well as their contribution to the anadromous brown trout (sea trout) stock that forage in the Moy Estuary.

### 3. Material and Methods

Biological material for the Moy project genetic analysis was provided by Inland Fisheries Ireland, the Lough Conn and Cullin Anglers Association & Mr. Judd Ruane (sea trout fishing charter). It 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. Fish sampling from both rivers and lakes was carried out by IFI, following Standard Operating Procedures (SOP's) established by the IFI for electrofishing in wadeable rivers and lake surveys (IFI unpublished).

#### 3.1 River Samples

River samples for the identification of baseline river populations consisted of 1,286 juveniles (<17 cm in fork length and 0+ & 1+ in age) in addition to 455 adult (fish  $\geq 17$ cm in length) brown trout sampled from 80 sites (2 to 47 individuals per site, avg.  $\sim 20$ ) and 32 rivers across the area of study. In each case, non-invasive biopsy tissue (scales) was removed from brown trout (0+, 1+ juveniles and adults). These were sampled by electrofishing from the main river sub-catchments of the Moy, Palmerstown and Easkey between 2012 and 2016. A minimum of two sites were surveyed within each sub-catchment. To reduce the possibility of sampling siblings (family bias), at each site, individual samples were gathered across a section of the river. Criteria for the selection of sampling location was based on previously collected data from electrofishing, redd counts, nursery mapping, and habitat surveys, collected over many years as part of the IFI ongoing monitoring river surveys (IFI unpublished data, O'Grady *et al.*, 1994, O'Grady *et al.*, 1997 & 1998, O'Grady & Delanty 2001). The geographical locations of all sampling sites are shown in Figure 3 and detailed in Table 1.



**Figure 3.** Locations of brown trout sampling in the Moy catchment. In each case, pie size is proportional to number (also shown) of individuals non-destructively sampled per site. The River Moy is highlighted in deep purple colour.

**Table 1.** Number and location of fish (juveniles and adults) sampled from rivers, lakes, and estuary used in this study. To facilitate identification of sample location, samples from the larger Moy Catchment were categorized by main regions (i.e., East, West and North). Those on the western side were further categorized into the lake they flow into (Lough Conn or Lough Cullin). Farm fish (Roscrea and Leven origin) samples are also included as a genetic baseline reference for hatchery derived fish. NA – indeterminate age.

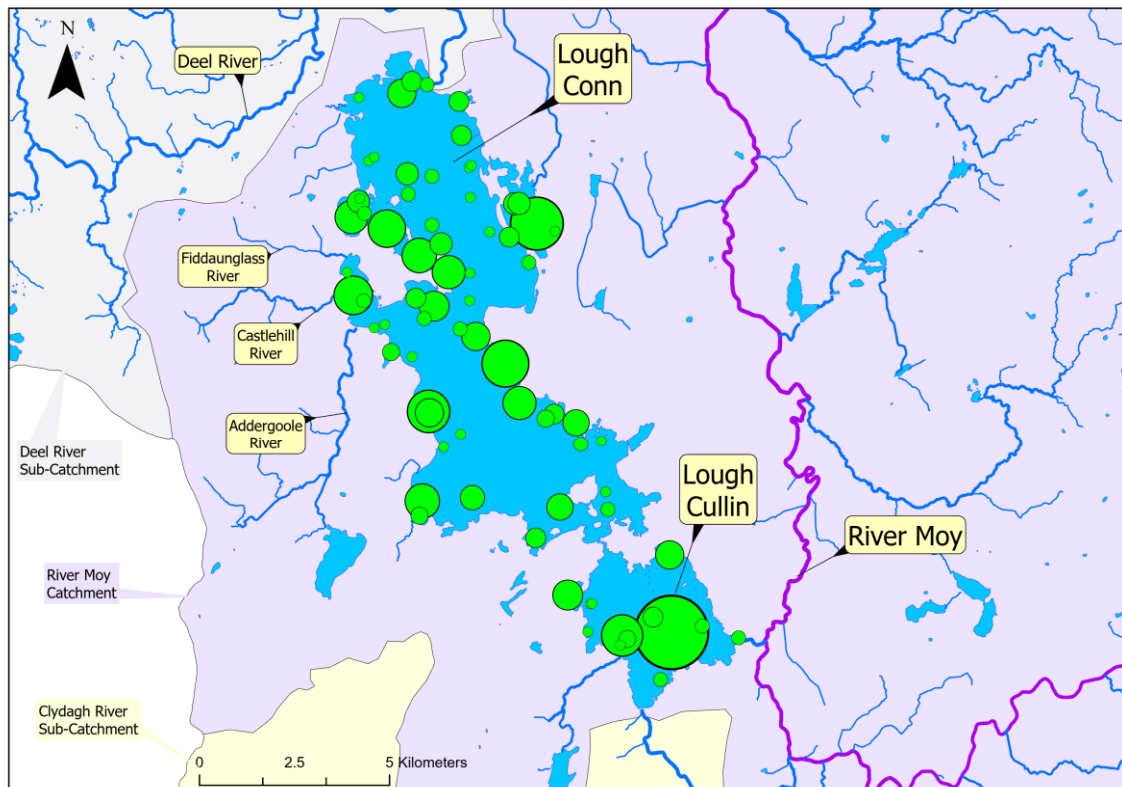
Sample Location	Source	Flows into	Region	Catchment	Juvenile	Adult	NA	Total
Deel	1	Lough Conn	Western Moy	Deel	73	21		94
Addergoole	1	Lough Conn	Western Moy	Moy	118	15		133
Brandra	1	Lough Conn	Western Moy	Moy	25	17		42
Fiddaunglass	1	Lough Conn	Western Moy	Moy	69	18	1	88
Fortland	1	Lough Conn	Western Moy	Moy	42	4		46
Massbrook South	1	Lough Conn	Western Moy	Moy	38	4		42
Castlehill	1	Lough Conn	Western Moy	Moy	83	4		87
Tobergal	1	Lough Cullin	Western Moy	Moy	33	5		38
Castlebar River	1	Lough Cullin	Western Moy	Castlebar	16	28		44
Clydagh	1	Lough Cullin	Western Moy	Clydagh	60	21		81
Manulla	1	Lough Cullin	Western Moy	Manulla	14	26		40
Clonlea/Cloonlee	1	River Moy	Eastern Moy	Moy	29	12		41
Einagh	1	River Moy	Eastern Moy	Moy	65	11		76
Killeen	1	River Moy	Eastern Moy	Moy	18	24		42
Mad River	1	River Moy	Eastern Moy	Moy	15	2		17
Mullaghanoe	1	River Moy	Eastern Moy	Moy	56	22		78
Owenaher	1	River Moy	Eastern Moy	Moy	34	6		40
Sonnagh	1	River Moy	Eastern Moy	Moy	19	30		49
Spaddagh	1	River Moy	Eastern Moy	Moy	20	24		44
Straide	1	River Moy	Eastern Moy	Moy	20	24		44
Swinford	1	River Moy	Eastern Moy	Moy	21	18		39
Yellow River (Foxford)	1	River Moy	Eastern Moy	Moy	32	15		47
Glore	1	River Moy	Eastern Moy	Gweestion	32	24	3	59
Pollagh	1	River Moy	Eastern Moy	Gweestion	19	10		29
Yellow River	1	River Moy	Eastern Moy	Gweestion	25	8		33
Owengarve	1	River Moy	Eastern Moy	Owengarve	39	6		45
Moy Main Channel Upper	1	River Moy	Eastern Moy	Moy	64	22		86
Trimogue	1	River Moy	Eastern Moy	Trimoge	17	3		20
Bunree	1	River Moy (Tidal)	North Moy	Brusna	73	4		77
Glenree	1	River Moy (Tidal)	North Moy	Brusna	14			14
Palmerstown	1	Moy Estuary	North Moy	Palmerstown	29	9	4	42
Easkey	1	Atlantic Ocean	-	Easkey	74	18		92
L. Conn	2	Lough Cullin	-	-	1	332		333
L. Cullin	2	River Moy	-	-		116		116
Moy Estuary	3	Atlantic Ocean	North Moy	Moy	3	83	2	88
Farm fish	-	-	-	-		110		110
<b>Total</b>					<b>1290</b>	<b>1096</b>	<b>10</b>	<b>2396</b>

### 3.2 Lake Samples

Lake adult brown trout (mixed fishery) were collected by both IFI and the Lough Conn and Cullin Anglers Association between 2011 and 2013. Samples from Lough Conn were collected in 2013 by IFI during Research lake fish stock assessments using survey gillnets set at different depths throughout the lake (random survey method - O'Grady & Delanty 2001). Samples provided by anglers were collected between 2011 and 2013 angling seasons from both Lough Conn and Lough Cullin. A total of 332 adult brown trout from Lough Conn and 116 from Lough Cullin were collected and available for



analysis (see Table 1 and Fig. 4 for details). Where possible details such as fish length, weight and location were also collected and stored on a central database.



**Figure 4.** Site location of adult brown trout sampled from Lough Conn and Lough Cullin examined in this study. In each case, pie size is proportional to number of individuals (ranging from 1 to 29 avg. ~5) non-invasively sampled per site. A subset of adult brown trout from Lough Cullin, consisting of 56 individuals for which there was no GPS data, were arbitrarily placed at the centre of the lough.

### 3.2 Sea trout Samples

Sea trout samples (N =88) were provided by sea trout fishing charters in 2014 (N=40) and 2015 (N=48). A detailed map of the Moy estuary indicating the general sampling area for sea trout is included in Figure 13.

### 3.3 Genetic Analyses (DNA Profiling)

Genomic DNA was extracted from all brown trout specimens using the Promega Wizard SV 96 genomic DNA purification kit. The extracted DNA was then transferred into 96 well microtiter plates and stored at -20°C for future genetic analysis. All samples were screened (i.e., genotyped) for a marker panel comprising 20 microsatellite loci (*Ssa85*, *One102a*, *One102b*, *One108*, CA054565, *Ssa416*, *One103*, *Cocl-Lav-4*, *One9ASC*, CA048828, CA053293, BG935488, *SsaD71*, *SaSaTAP2A*, MHC1, *Ssa410UOS*, *ppStr2*, *ppStr3*, CA060177 and *Ssa197*) developed and/or optimised at QUB for brown trout population genetic studies (Keenan *et al.*, 2013a). Using protocols developed and routinely used at QUB, genetic screening was conducted on a 96 capillary ABI 3730XL DNA analyser. The marker selection criteria and genetic screening protocols are outlined in Keenan *et al.* (2013a). The genotypic data obtained was compiled into an Excel database for subsequent statistical genetic analyses.

### 3.4 Farm brown trout samples

The analysis also included microsatellite genotypic data from 110 fish of farm origin (Roscrea and Leven). This dataset was sourced from previous population genetic studies on brown trout conducted by IFI and QUB (Delanty *et al.*, 2021 & 2022, Hynes *et al.*, 2022), using the same panel of microsatellite markers. It served as the baseline genetic reference for the identification of farmed stocked fish.

### 3.5 Statistical Analysis

#### 3.5.1 Identification of Baseline populations

Following genotyping, only high-quality data was included in the subsequent statistical analysis. Thus, individual brown trout that could not be unambiguously scored for at least 14 of the 20 microsatellite loci (70% of scored loci per individual fish) were removed from the main data set. To prevent possible biases for the identification of baseline populations, individuals caught in the same site were checked for the presence of full siblings using the Colony v2.0.6.2 program (Jones and Wang, 2010). If more than three individuals were identified as siblings, only three (randomly selected) were retained for analyses (Waples & Anderson 2017).

The baseline river genetic data was analysed for population genetic structure using the Bayesian framework of STRUCTURE V2.4 (Pritchard *et al.*, 2000) and employing the hierarchical approach proposed by Rosenberg *et al.* (2002). This approach allows for the identification of major genetic clusters within the dataset that are potentially linked by a shared ancestry, followed by additional partitioning of these major clusters into populations (i.e., genetic baselines). To ensure an unbiased sample data set for the examination of the brown trout population genetic structuring within the larger River Moy catchment (i.e. definition of the baseline river populations), adult fish (fish  $\geq 17$ cm in length) were excluded from the STRUCTURE analysis. These “unknown” adults were subsequently used as part of the adult mixed stock to assist in the validation of individual population assignment. The rationale was that a large proportion of these adult fish should assign to populations linked to the rivers where they were originally captured.

STRUCTURE was first run with all samples, including the farm fish baseline (Roscrea and Leven origin), to investigate the potential impact of stocking on the genetic composition of the brown trout populations inhabiting the larger River Moy catchment system. The farm fish samples were removed from subsequent STRUCTURE analysis that aimed to assess the patterns of contemporary population structuring within the system. To further investigate the genetic relationships among the inferred brown trout populations within the River Moy catchment, a neighbour-joining (NJ) phylogenetic tree, based on Nei’s DA (Nei *et al.*, 1983), was constructed using POPTREE2 (Takezaki *et al.*, 2010).

Summary statistics, including the number of alleles per locus, allelic richness (AR), observed and expected heterozygosity, and tests for conformity to Hardy-Weinberg (HW) expectations, were calculated for the baseline populations inferred by STRUCTURE using the *divBasic* function from the *diveRsity* package (Keenan *et al.*, 2013b). Pairwise F-statistics, including both  $\theta$  (Weir & Cockerham, 1984) and  $D_{Jost}$  (Jost, 2008) were estimated between all populations identified by the STRUCTURE analysis using the *fastDivPart* function of *diveRsity*. The statistical significance of estimated values was evaluated both by bootstrapping (1000) using *diveRsity*.

### 3.5.2 Genetic Stock Identification

To evaluate the power and usefulness of the population genetic baseline inferred by STRUCTURE for individual assignment, that is, to assign an adult individual to the correct population baseline, the self-assignment test of the program ONCOR (Kalinowski *et al.*, 2007) was used. ONCOR was also used to subsequently assign lake caught adult brown trout to the STRUCTURE inferred populations, with an “ad-hoc” assignment *P* value of  $\geq 0.7$  being considered as robust (i.e. biologically meaningful) assignments (Prodöhl *et al.*, 2017). The farm fish baseline (Roscrea and Leven origin) was also included in this analysis.

#### 4. Results

In total, 2,286 specimens were collected for this study, comprising 1,290 juveniles, 986 adults, and 10 specimens of indeterminate age. Genetic screening of these samples revealed the presence of 74 (3.04%) Atlantic salmon, *Salmo salar*, individuals among the juvenile samples, which were excluded from further analyses. While 44 (1.9%) samples were also excluded from the analysis due to contamination issues, an additional 162 samples were also removed as they did not meet the minimum genetic criteria for inclusion, specifically, having complete genotypic data for 14 or more loci. In summary, high-quality genetic data were successfully obtained for the following: 1,392 specimens (86%) from the Moy Catchment and its sub-catchments; 84 specimens (91%) from the Easkey catchment; 38 specimens (90%) from the Palmerstown catchment; 75 specimens (85%) from the Moy Estuary; and 417 specimens (93%) from L. Conn and L. Cullin. A summary of the samples used in subsequent statistical analyses is presented in Table 2.

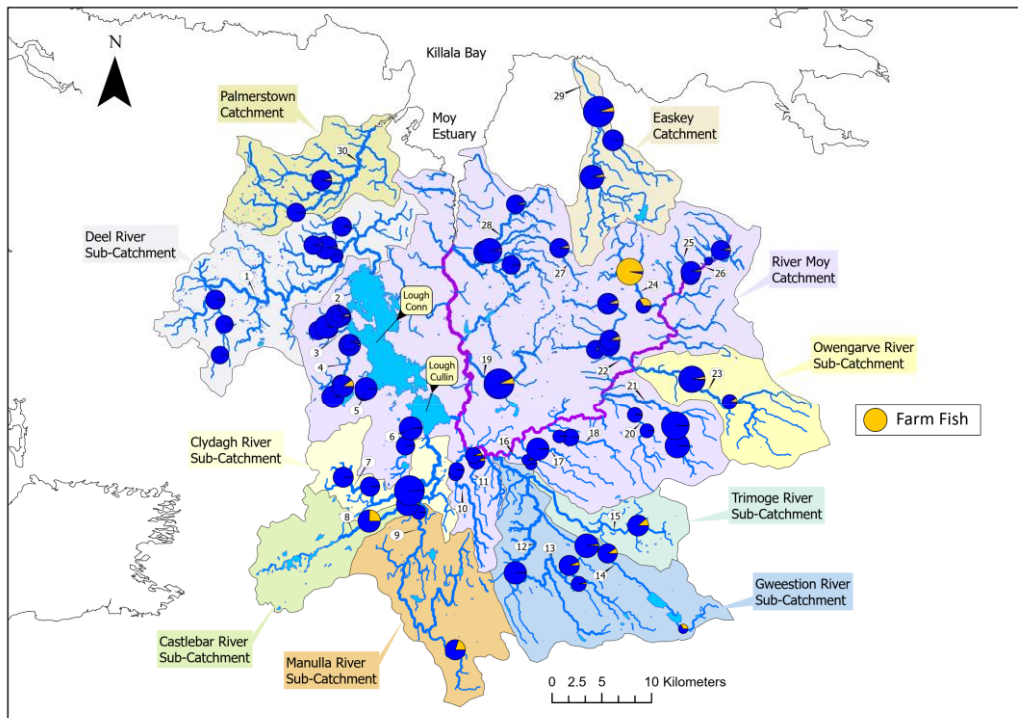
**Table 2.** Number of samples caught and used in the analyses after the application of the sample quality control criteria (i.e. only individuals scored  $\geq 14$  loci were used for statistical analyses). The percentage of sample retention is also provided. \* “Broad Moy” comprises all samples caught in the main Moy Catchment and sub-catchments.

Catchment	Collected	Analysed	%
Broad Moy*	1,615	1,392	86
Easkey	92	84	91
Palmerstown	42	38	90
Estuary	88	75	85
Lake	449	417	93

The hierarchical STRUCTURE analysis results, illustrated in Fig. 5, summarise the impact of stocking on wild river brown trout of the Moy Catchment. The genetic data is most effectively explained by two distinct groups: wild-origin brown trout (depicted in blue) and hatchery/farm-derived brown trout (in orange). Overall, there is no substantial evidence indicating significant mixing of hatchery/farm-derived genes into wild brown trout. However, a notable exception emerged in the sample collected from the Upper Owenaher River (as shown in Figure 5), where most of the 24 brown trout specimens appear to be of farm genetic origin. In addition, some evidence of farm-derived fish was also observed in samples taken from the Castlebar River (Figure 5).

The results of the subsequent levels of the hierarchical STRUCTURE analysis, excluding brown trout of farm origin, are summarised in Figures 6 to 10. In the first hierarchical level (shown in Fig. 6), samples are explained by three genetic clusters. The first group (shown in blue colour) comprises samples from the upper reaches of the Owenaher River. The second group (in purple) includes samples from the Palmerstown and Easkey catchments, along with those representing the Brusna River catchment (Bunree & Glenree). Interestingly, most of the samples from the Clydagh River also fall into this group. The remaining samples belong to the third genetic group (illustrated in green). Excluding the first group, it is noteworthy to observe the geographical pattern of mixture between the second (purple) and third (green) genetic groups. Thus, the second group appears to be more common in the Eastern (rivers flowing into the main River Moy channel) and South Western (rivers flowing into Lough Cullin) regions of the Moy catchment, with relatively fewer representatives in the Western region (rivers

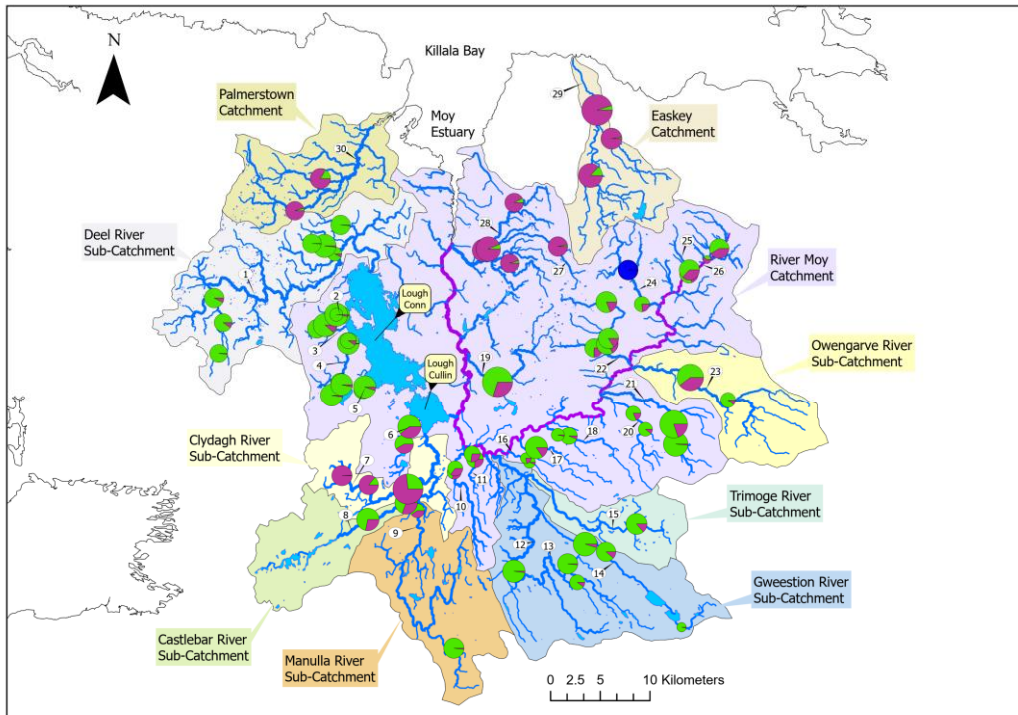
flowing into Lough Conn). This latter region is predominantly occupied by samples belonging to the second group (green).



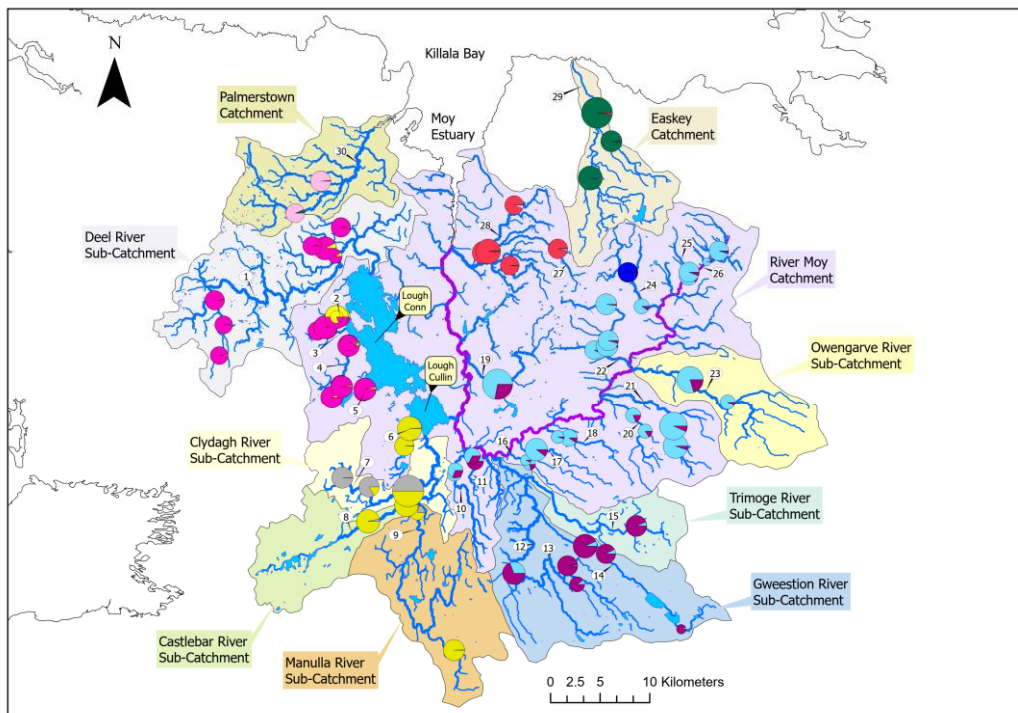
**Figure 5.** Pie charts summarising the results obtained from STRUCTURE analysis, highlighting genetic differences between brown trout collected from the Moy catchment system (including both Palmerstown and Easkey neighbour catchments) and farm brown trout (originating from Roscrea and Leven). Each distinct genetic group is shown using a different colour, where blue and orange represent wild and farm origin, respectively. The size of each pie chart corresponds to the number of samples collected from each location, while the slices within each pie chart indicate the proportion of individuals with a specific genetic background in the sample. The River Moy is highlighted in deep purple colour. The main rivers sampled in the study are listed numerically, including: 1- Deel; 2- Fiddaunglass; 3- Castlehill; 4- Addergoole; 5- Massbrook; 6- Tobergal; 7- Clydagh; 8- Castlebar; 9- Manulla; 10- Straide; 11- Clonlea; 12- Pollagh; 13- Yellow; 14- Glore; 15- Trimogue; 16- Killeen; 17- Spaddagh; 18- Swinford; 19- Yellow (Foxford); 20- Sonnagh; 21- Mullaghano; 22- Einagh; 23- Owengarve; 24- Owenaher; 25- Mad; 26- Moy Main Channel (Upper); 27- Glenree; 28- Brusna; 29- Easkey; 30- Palmerstown.

In the second level of hierarchical STRUCTURE analysis (shown in Fig. 7), samples are further partitioned in well-defined geographical groups. Thus, all samples from Western region (rivers flowing into Lough Conn) belong to the same ancestral genetic group. With the exception of Clydagh River catchment, all the other samples from rivers flowing into Lough Cullin belong to a second ancestral group while a third group comprises samples from both the Gweestion and Trimogue rivers' sub-catchment that flow into the River Moy main channel. A fourth group was composed of all other samples from the Moy catchment that flow into the Moy River main channel with the exception of samples from the Brusna River that form an independent group in the north region of the Moy catchment. At this level of the hierarchical analysis, samples from both the Palmerstown and Easkey catchments are also fully differentiated from other locations.

This well-defined geographical partitioning of groups down to populations is also evident in the third (shown in Fig. 8), fourth (shown in Fig. 9) and the final fifth (shown in Fig. 10) levels of the hierarchical STRUCTURE analysis. No further statistically significant partitioning was observed following this last level of the analyses.

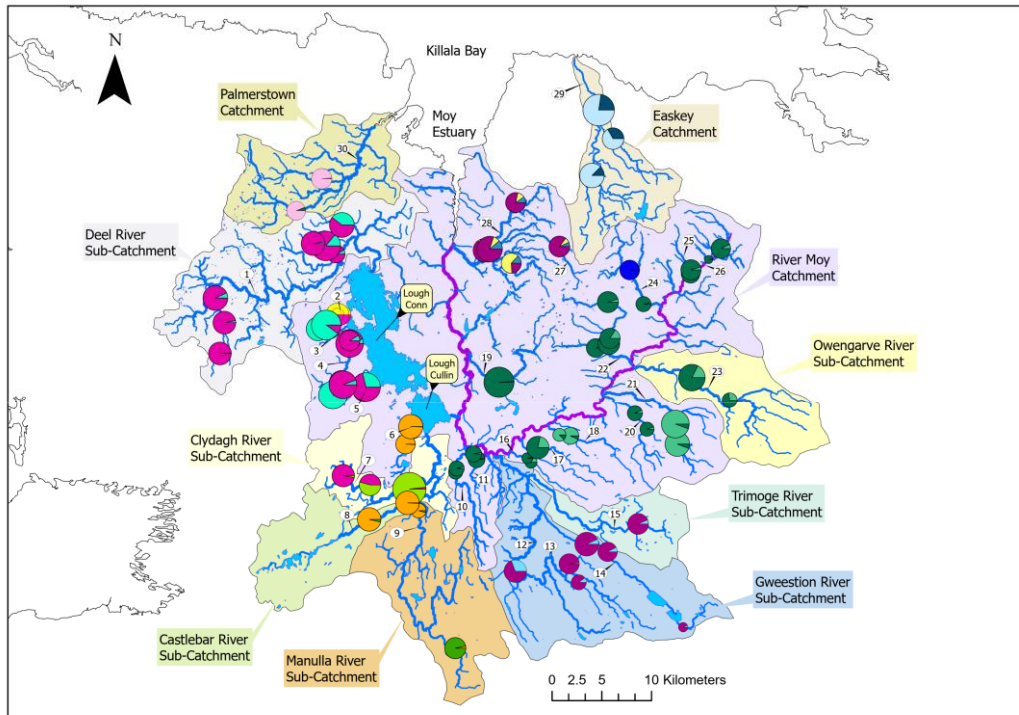


**Figure 6.** Pie charts summarising the results of the first level of the STRUCTURE analysis (excluding farm derived samples). Each distinct genetic group is shown using a different colour. The size of each pie chart corresponds to the number of samples collected from each location, while the slices within each pie chart indicate the proportion of individuals with a specific genetic background in the sample. See legend of Fig. 5 for numerical labelling of the main rivers sampled in the study. The River Moy is highlighted in a deep purple colour.

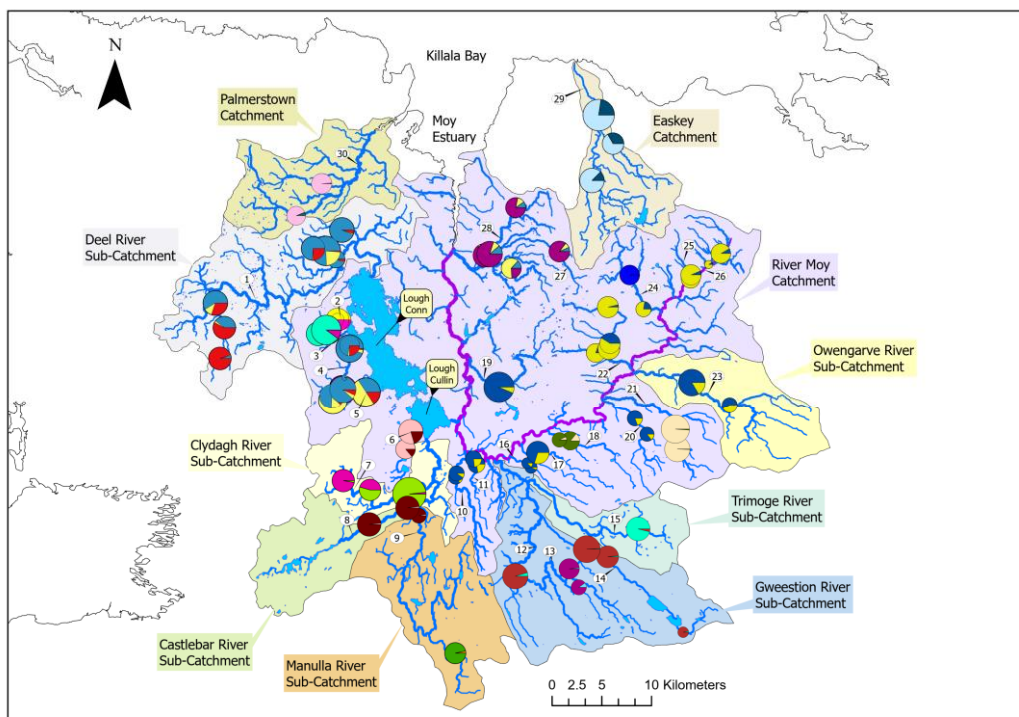


**Figure 7.** Pie charts summarising the results of the second level of the STRUCTURE analysis. Each distinct genetic group is shown using a different colour. The size of each pie chart corresponds to the number of samples collected from each location, while the slices within each pie chart indicate the proportion of individuals with a specific genetic background in the sample. See legend of Fig. 5 for numerical labelling of the main rivers sampled in the study. The River Moy is highlighted in a deep purple colour.

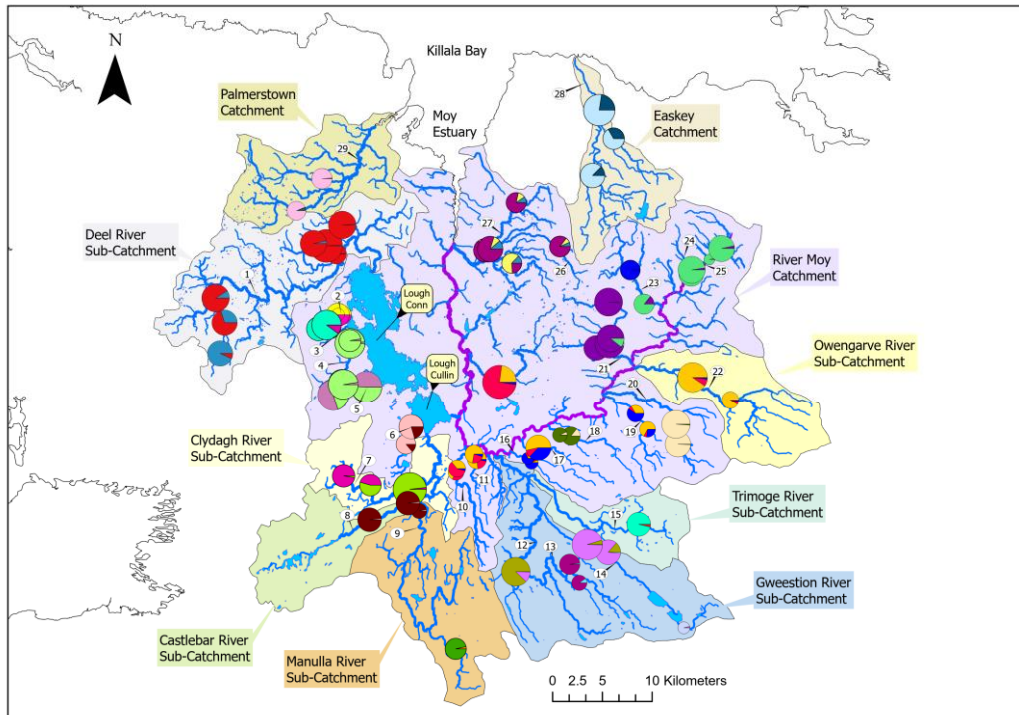




**Figure 8.** Pie charts summarising the results of the third level of the STRUCTURE analysis. Each distinct genetic group is shown using a different colour. The size of each pie chart corresponds to the number of samples collected from each location, while the slices within each pie chart indicate the proportion of individuals with a specific genetic background in the sample. See legend of Fig. 5 for numerical labelling of the main rivers sampled in the study. The River Moy is highlighted in a deep purple colour.



**Figure 9.** Pie charts summarising the results fourth level of the STRUCTURE analysis. Each distinct genetic group is shown using a different colour. The size of each pie chart corresponds to the number of samples collected from each location, while the slices within each pie chart indicate the proportion of individuals with a specific genetic background in the sample. See legend of Fig. 5 for numerical labelling of the main rivers sampled in the study. The River Moy is highlighted in a deep purple colour.



**Figure 10.** Pie charts summarising the results of the fifth level of the STRUCTURE analysis. Each distinct genetic group is shown using a different colour. The size of each pie chart corresponds to the number of samples collected from each location, while the slices within each pie chart indicate the proportion of individuals with a specific genetic background in the sample. See legend of Fig. 5 for numerical labelling of the main rivers sampled in the study. The River Moy is highlighted in a deep purple colour.

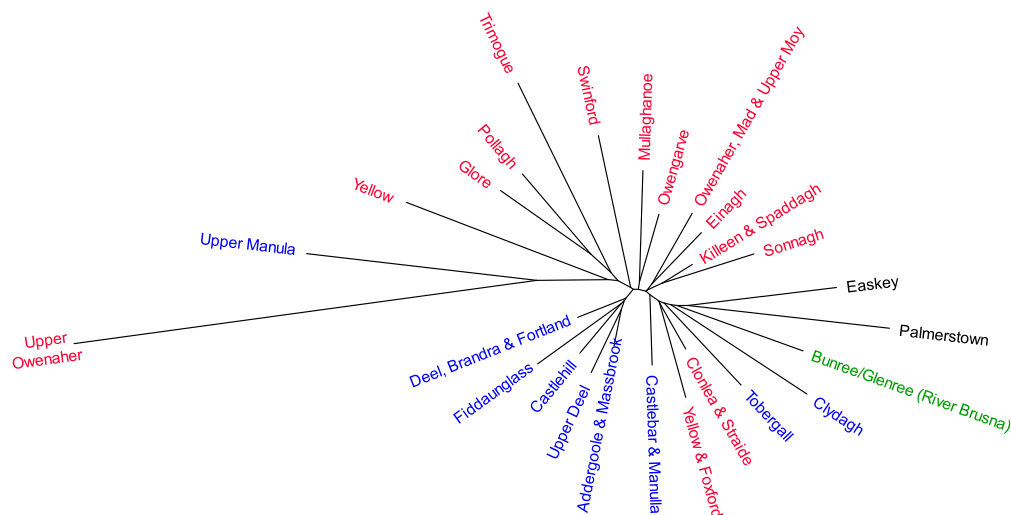
From the STRUCTURE analysis, 26 genetically distinct reporting units were identified, each consisting of one or more baseline river populations (Table 3). Western Moy contains nine of these units, while Eastern Moy has 14, and North Moy contains one. Two additional groups are individually represented by Easkey and Palmerstown. Summary statistics (e.g. observed and expected heterozygosity, samples size, allele richness) for these reporting groups/populations are also presented in Table 3.

The inferred reporting groups/populations exhibited average allele richness values ranging between 3.6 (Upper Owenaher) and 7.4 (Clonlea & Straide), with a mean of 6.2. With one exception, both observed (average 0.65) and expected heterozygosity (average 0.66) values were consistently similar across samples. Not surprising, Upper Owenaher, however, deviated from this trend, displaying lower observed and expected heterozygosity rates of 0.49 and 0.47.

Nei's DA neighbour-joining (NJ) phylogenetic tree (Fig. 11) largely corroborate population structuring observed in the STRUCTURE analysis. Thus, with a few exceptions, there is a clear split between populations from western, eastern and north of the catchment. Some populations' unusual placements in the tree (e.g. Clydagh and Tobergal) in terms of geography, are also reflected in the STRUCTURE analysis. However, the unusual positions of both the Upper Manulla and Upper Owenaher can be probably attributed to their limited sample sizes, which make them more susceptible to genetic drift. Another point of interest is the generally weak support for the nodes (all below 50% bootstrap support – not shown) in the tree, suggesting low genetic divergence among the populations.

**Table 3.** Genetically distinct reporting groups/populations identified by STRUCTURE analysis, and related summary statistics. N = number of samples that have amplified for over 70% of loci and used for data analyses; Ar = average allelic richness; Ho and He = observed and expected heterozygosity.

Reporting Groups	Region	N	Ar	Ho	He
1. Upper Deel	Western Moy	57	6.8	0.64	0.67
2. Deel, Brandra & Fortland	Western Moy	75	6.8	0.65	0.68
3. Fiddaunglass	Western Moy	82	6.0	0.63	0.66
4. Castlehill	Western Moy	69	6.9	0.68	0.69
5. Addergoole & Massbrook	Western Moy	128	7.2	0.67	0.69
6. Tobergall	Western Moy	37	6.9	0.67	0.69
7. Clydagh	Western Moy	79	6.3	0.6	0.64
8. Castlebar & Manulla	Western Moy	59	7.1	0.66	0.69
9. Upper Manulla	Western Moy	15	4.4	0.63	0.61
10. Clonlea & Straide	Eastern Moy	116	7.4	0.66	0.7
11. Pollagh	Eastern Moy	28	5.9	0.64	0.63
12. Yellow	Eastern Moy	31	5.4	0.62	0.63
13. Glore	Eastern Moy	50	5.7	0.64	0.64
14. Trimogue	Eastern Moy	19	5.4	0.64	0.64
15. Killeen & Spaddagh	Eastern Moy	71	6.9	0.67	0.68
16. Swinford	Eastern Moy	38	5.8	0.63	0.62
17. Yellow (Foxford)	Eastern Moy	43	6.5	0.66	0.66
18. Sonnagh	Eastern Moy	45	6.6	0.69	0.7
19. Mullaghanoe	Eastern Moy	65	6.3	0.64	0.67
20. Einagh	Eastern Moy	72	7.2	0.68	0.69
21. Owengarve	Eastern Moy	39	6.7	0.66	0.68
22. Upper Owenaher	Eastern Moy	27	3.6	0.49	0.47
23. Owenagher, Mad & Upper Moy	Eastern Moy	26	6.4	0.68	0.69
24. Bunree/Glenree (River Brusna)	North Moy	83	6.3	0.63	0.66
25. Easkey	-	84	6.2	0.64	0.65
26. Palmerstown	-	38	5.7	0.66	0.66



**Figure 11.** Unrooted NJ phylogenetic tree based on Nei's DA (1983) genetic distance illustrating the relationship among the River Moy catchment inferred brown trout populations. Blue: Western Moy; Red: Eastern Moy; Green: North Moy.

The inferred reporting groups/populations from the Moy catchment system exhibited an overall population divergence level of  $F_{ST} = 0.062$  (95% C.I. 0.059 - 0.065). This finding aligns well with observations from brown trout populations in other systems both in Ireland and internationally, as noted in studies by Prodöhl *et al.* (2019), Delanty *et al.* (2020, 2022), and Hynes *et al.* (2022). The pairwise  $F_{ST}$  and  $D_{Jost}$  estimates, along with their 95% CI, which assess statistical significance, for all 26 populations inferred by STRUCTURE are displayed in Table 4. A strong positive correlation ( $r = 0.98$ ) was evident between both genetic differentiation metrics. Within the River Moy system, the most genetically distinct populations or reporting groups included Upper Owenaher, Upper Manulla, Clydagh, Yellow, Trimogue, and Brusna. The Easkey and Palmerstown populations also displayed significant divergence when contrasted with the other populations analysed in this study.

The results of the self-assignment test that was used to assess the power and usefulness of the baseline populations inferred by STRUCTURE for individual assignment (i.e., to assign an adult individual to the correct population baseline) are displayed in Table 5. The inferred baseline populations/reporting groups exhibit an average self-assignment rate of 73%, underscoring the effectiveness and relevance of the established baseline populations/reporting groups in assigning individuals with unknown origins. However, considerable variation in self-assignment rates was noted among individual populations/reporting units, with values ranging from 44% in the Owengarve River to a full 100% in Owenaher (lower), Mad & Upper Moy reporting group, as well as, farm derived samples.

Low self-assignment rates are often related with low genetic differentiation and high levels of gene flow among populations. Notably, in several cases, such as populations associated with the Lough Conn and Lough Cullin catchments, mis-assigned individuals tend to be allocated to populations/reporting groups that are both genetically and geographically related. However, this pattern is not as evident for rivers that flow into the Main River Moy Channel, like the Straide, Clonlea, and Owengarve Rivers. Interestingly, the predominant mis-assignment observed for individuals from the Straide and Clonlea Rivers is towards the Owengarve River and vice-versa. In both scenarios, the Einagh River is also linked to these mis-assignments but not the other way around. Overall, it is important to highlight that there were no mis-assignments of wild derived samples to the Farm genetic baseline, and all farm samples were accurately assigned to the 'Farm' baseline.

Results of the assignment of river adult caught fish to reporting groups identified by the STRUCTURE analysis baselines are summarised in Table 6 (A & B). On average, ~78% of the adult fish caught in the rivers assigned to one of the baseline population/reporting groups with high confidence ( $P \geq 0.7$ ). There was a strong correlation between the capture location of adult fish and their reporting group. Thus, on average, over 84% of adults caught in a specific river were associated with the population or reporting group corresponding to that river. The few exceptions involved a small number of individuals, which often assigned to a population or reporting group geographically close to their capture site. It is important to highlight that several individuals assigning with "lower confidence" (i.e.  $P < 0.7$ ) were often associated with a population or reporting group linked to the river where they were caught or an adjacent one. This suggests that, even though these assignments were made with lower confidence, they are probably accurate. Thus, the figure presented in here (i.e. 78%) is likely to be a slight underestimation. Overall, these results reinforce the efficacy and relevance of the established baseline populations/reporting groups in determining the origin of unknown fish.

**Table 4.** Pairwise estimates of (A)  $F_{ST}$  and (B)  $D_{Jost}$  (below diagonal in each case) and associated 95% confidence intervals (above diagonal) illustrating genetic divergence among the inferred reporting groups/populations. The heatmap colour gradient represents the relative divergence levels in pair-wise comparisons. A “red” hue indicates greater genetic divergence between populations whereas “blue” represents lesser divergence. All pair-wise population comparisons were found to be statistically significant.

A) $F_{ST}$	Upper Deel	Deel, Brandra & Forland	Fiddauglass	Castlehill	Adergoole & Massbrook	Tobergall	Clydagh	Castlebar	Upper Manulla	Straide & Clonlea	Killeen	Pollagh	Yellow	Glore	Trimogue	Swinford	Sonnagh	Mullaghanoe	Owengarve	Einagh	Upper Owenaher	Owenagher, Mad & Upper Moy	Yellow (Foxford)	Brusna (Bunree/Glenree)	Easkey	Palmerstown
Upper Deel		0.008-0.021	0.027-0.048	0.014-0.031	0.007-0.019	0.014-0.034	0.054-0.085	0.019-0.037	0.006-0.095	0.01-0.024	0.009-0.023	0.015-0.039	0.059-0.097	0.032-0.056	0.041-0.074	0.038-0.066	0.023-0.043	0.026-0.044	0.013-0.033	0.017-0.031	0.16-0.195	0.023-0.039	0.018-0.038	0.048-0.067	0.077-0.096	0.079-0.108
Deel, Brandra & Forland	0.014		0.018-0.037	0.005-0.014	0.014-0.032	0.057-0.089	0.011-0.024	0.009-0.024	0.007-0.02	0.015-0.038	0.046-0.079	0.025-0.042	0.035-0.066	0.014-0.03	0.02-0.035	0.002-0.017	0.014-0.026	0.15-0.18	0.017-0.031	0.017-0.031	0.012-0.027	0.038-0.053	0.059-0.08	0.063-0.092	0.063-0.092	0.063-0.092
Fiddauglass	0.037	0.027		0.019-0.038	0.017-0.034	0.029-0.05	0.072-0.103	0.026-0.047	0.069-0.11	0.024-0.043	0.025-0.044	0.023-0.046	0.077-0.118	0.034-0.057	0.048-0.084	0.034-0.064	0.035-0.06	0.035-0.058	0.021-0.043	0.03-0.05	0.176-0.209	0.032-0.048	0.028-0.047	0.057-0.079	0.067-0.087	0.083-0.112
Castlehill	0.022	0.016	0.029		0.004-0.014	0.017-0.032	0.065-0.105	0.012-0.024	0.023-0.051	0.065-0.102	0.03-0.046	0.039-0.075	0.035-0.063	0.016-0.033	0.028-0.044	0.012-0.031	0.014-0.027	0.164-0.195	0.021-0.035	0.02-0.037	0.164-0.195	0.021-0.035	0.02-0.037	0.041-0.059	0.061-0.083	0.065-0.09
Adergoole & Massbrook	0.012	0.009	0.024	0.009		0.019-0.032	0.06-0.086	0.015-0.029	0.062-0.091	0.011-0.023	0.008-0.018	0.015-0.038	0.058-0.092	0.025-0.041	0.034-0.064	0.03-0.055	0.017-0.034	0.026-0.039	0.009-0.026	0.017-0.027	0.154-0.183	0.022-0.034	0.016-0.03	0.043-0.058	0.061-0.079	0.065-0.09
Tobergall	0.023	0.021	0.038	0.024	0.025		0.013-0.036	0.058-0.092	0.006-0.021	0.014-0.034	0.019-0.044	0.063-0.107	0.032-0.058	0.046-0.078	0.051-0.086	0.022-0.043	0.027-0.05	0.014-0.04	0.017-0.037	0.231-0.272	0.185-0.224	0.033-0.05	0.008-0.025	0.033-0.055	0.054-0.082	0.054-0.082
Clydagh	0.069	0.072	0.086	0.08	0.072	0.061		0.05-0.084	0.044-0.07	0.055-0.084	0.06-0.097	0.1-0.147	0.077-0.108	0.064-0.11	0.103-0.149	0.058-0.089	0.076-0.106	0.055-0.09	0.067-0.097	0.231-0.272	0.185-0.224	0.033-0.05	0.008-0.025	0.033-0.055	0.054-0.082	0.054-0.082
Castlebar	0.028	0.017	0.035	0.024	0.022	0.024	0.066		0.006-0.022	0.012-0.028	0.024-0.053	0.054-0.093	0.022-0.042	0.032-0.058	0.017-0.037	0.017-0.034	0.007-0.026	0.009-0.023	0.15-0.181	0.03-0.05	0.15-0.181	0.03-0.05	0.018-0.04	0.04-0.061	0.053-0.078	0.055-0.085
Upper Manulla	0.075	0.070	0.087	0.068	0.074	0.074	0.113	0.062		0.06-0.096	0.06-0.092	0.071-0.114	0.095-0.152	0.081-0.122	0.105-0.156	0.053-0.086	0.044-0.085	0.046-0.082	0.036-0.07	0.177-0.231	0.135-0.183	0.055-0.086	0.066-0.106	0.086-0.124	0.107-0.149	0.097-0.139
Straide & Clonlea	0.016	0.016	0.034	0.02	0.017	0.013	0.056	0.013	0.076		0.009-0.025	0.01-0.035	0.055-0.094	0.024-0.044	0.033-0.068	0.024-0.074	0.015-0.036	0.023-0.044	0.008-0.03	0.111-0.026	0.167-0.206	0.102-0.026	0.016-0.032	0.044-0.063	0.063-0.085	0.074-0.101
Killeen	0.015	0.013	0.033	0.018	0.013	0.023	0.069	0.019	0.074	0.016		0.02-0.045	0.05-0.083	0.03-0.05	0.029-0.057	0.007-0.023	0.02-0.036	0.006-0.025	0.009-0.023	0.162-0.193	0.106-0.139	0.016-0.03	0.014-0.032	0.044-0.063	0.063-0.085	0.074-0.101
Pollagh	0.026	0.025	0.033	0.036	0.026	0.031	0.078	0.038	0.092	0.021	0.031		0.064-0.114	0.024-0.062	0.046-0.092	0.048-0.085	0.031-0.065	0.039-0.07	0.022-0.054	0.203-0.251	0.152-0.195	0.022-0.054	0.016-0.032	0.044-0.063	0.062-0.097	0.078-0.117
Yellow	0.077	0.062	0.097	0.083	0.074	0.085	0.123	0.073	0.123	0.074	0.066	0.089		0.074-0.114	0.075-0.132	0.087-0.136	0.048-0.081	0.063-0.098	0.044-0.078	0.057-0.092	0.206-0.26	0.152-0.195	0.057-0.092	0.08-0.116	0.088-0.124	0.121-0.174
Glore	0.043	0.033	0.045	0.037	0.032	0.044	0.092	0.031	0.099	0.033	0.039	0.041	0.093		0.047-0.095	0.049-0.081	0.036-0.058	0.045-0.067	0.026-0.047	0.034-0.053	0.183-0.222	0.036-0.054	0.031-0.055	0.057-0.079	0.075-0.099	0.086-0.115
Trimogue	0.056	0.049	0.064	0.055	0.046	0.060	0.085	0.046	0.111	0.049	0.051	0.068	0.101	0.068		0.057-0.106	0.044-0.082	0.055-0.092	0.038-0.075	0.049-0.086	0.189-0.237	0.045-0.081	0.045-0.086	0.066-0.11	0.074-0.108	0.078-0.12
Swinford	0.051	0.039	0.048	0.048	0.042	0.057	0.125	0.045	0.129	0.057	0.042	0.065	0.110	0.063	0.078		0.044-0.077	0.025-0.057	0.04-0.07	0.17-0.208	0.042-0.068	0.045-0.079	0.072-0.106	0.084-0.119	0.106-0.141	0.106-0.141
Sonnagh	0.033	0.021	0.047	0.024	0.024	0.031	0.074	0.026	0.068	0.024	0.014	0.047	0.064	0.061	0.060	0.061		0.02-0.038	0.01-0.029	0.016-0.034	0.167-0.197	0.016-0.034	0.021-0.041	0.042-0.061	0.061-0.089	0.07-0.1
Mullaghanoe	0.034	0.027	0.046	0.036	0.032	0.038	0.090	0.025	0.062	0.033	0.028	0.054	0.080	0.055	0.072	0.059	0.028		0.014-0.032	0.014-0.032	0.156-0.19	0.023-0.041	0.032-0.054	0.053-0.077	0.087-0.111	0.084-0.115
Owengarve	0.023	0.009	0.031	0.021	0.017	0.026	0.072	0.015	0.062	0.018	0.015	0.036	0.060	0.035	0.055	0.040	0.018	0.022		0.008-0.025	0.129-0.165	0.009-0.026	0.015-0.038	0.026-0.054	0.051-0.076	0.068-0.105
Einagh	0.024	0.019	0.040	0.02	0.022	0.026	0.081	0.015	0.051	0.018	0.015	0.035	0.074	0.043	0.066	0.054	0.017	0.019	0.015		0.152-0.18	0.009-0.021	0.021-0.039	0.041-0.055	0.064-0.083	0.066-0.091
Upper Owenaher	0.177	0.164	0.192	0.179	0.169	0.203	0.251	0.166	0.202	0.187	0.177	0.226	0.233	0.202	0.211	0.188	0.182	0.173	0.146	0.165		0.141-0.177	0.095-0.236	0.097-0.256	0.234-0.26	0.226-0.273
Owenagher, Mad & Upper Moy	0.030	0.024	0.039	0.028	0.028	0.020	0.068	0.020	0.068	0.018	0.023	0.037	0.073	0.044	0.061	0.054	0.024	0.031	0.016	0.015	0.158		0.015-0.031	0.038-0.054	0.06-0.082	0.068-0.092
Yellow (Foxford)	0.027	0.019	0.037	0.028	0.023	0.015	0.062	0.028	0.084	0.011	0.023	0.027	0.077	0.043	0.063	0.061	0.030	0.042	0.025	0.029	0.214	0.022		0.033-0.049	0.053-0.076	0.057-0.083
Brusna (Bunree/Glenree)	0.057	0.045	0.067	0.05	0.050	0.043	0.079	0.050	0.102	0.042	0.053	0.070	0.097	0.068	0.081	0.088	0.051	0.062	0.040	0.047	0.222	0.046	0.041		0.069-0.09	0.067-0.092
Easkey	0.083	0.068	0.077	0.071	0.070	0.067	0.086	0.060	0.126	0.055	0.073	0.078	0.105	0.087	0.090	0.101	0.074	0.098	0.063	0.073	0.247	0.070	0.064	0.079		0.079-0.102
Palmerstown	0.092	0.077	0.097	0.076	0.077	0.066	0.118	0.069	0.117	0.073	0.087	0.096	0.146	0.099	0.098	0.123	0.084	0.100	0.086	0.078	0.248	0.079	0.069	0.078	0.090	
B) $D_{Jost}$	Upper Deel	Deel, Brandra & Forland	Fiddauglass	Castlehill	Adergoole & Massbrook	Tobergall	Clydagh	Castlebar	Upper Manulla	Straide & Clonlea	Killeen	Pollagh	Yellow	Glore	Trimogue	Swinford	Sonnagh	Mullaghanoe	Owengarve	Einagh	Upper Owenaher	Owenagher, Mad & Upper Moy	Yellow (Foxford)	Brusna (Bunree/Glenree)	Easkey	Palmerstown
Upper Deel		0.011-0.04	0.047-0.087	0.029-0.069	0.009-0.035	0.032-0.076	0.099-0.158	0.036-0.075	0.106-0.174	0.022-0.055	0.014-0.046	0.024-0.07	0.102-0.169	0.059-0.111	0.063-0.131	0.067-0.123	0.043-0.091	0.048-0.092	0.028-0.075	0.034-0.073	0.249-0.32	0.046-0.083	0.022-0.066	0.085-0.128	0.174-0.23	0.13-0.2
Deel, Brandra & Forland	0.025		0.03-0.066	0.013-0.039	0.003-0.021	0.022-0.058	0.094-0.152	0.022-0.053	0.091-0.159	0.015-0.043	0.011-0.036	0.022-0.061	0.079-0.135	0.041-0.078	0.047-0.104	0.05-0.11	0.023-0.059	0.033-0.065	0.001-0.035	0.029-0.058	0.193-0.256	0.03-0.065	0.01-0.041	0.081-0.113	0.111-0.155	0.11-0.174
Fiddauglass	0.066	0.047		0.039-0.077	0.033-0.066	0.059-0.11	0.131-0.193	0.044-0.087	0.127-0.198	0.049-0.089	0.052-0.09	0.034-0.072	0.113-0.183	0.057-0.098	0.083-0.152	0.058-0.103	0.069-0.11	0.056-0.095	0.032-0.075	0.066-0.11	0.243-0.295	0.054-0.086	0.045-0.084	0.107-0.149	0.125-0.164	0.157-0.217
Castlehill	0.048	0.026	0.057		0.008-0.028	0.04-0.08	0.136-0.195	0.028-0.076	0.112-0.175	0.019-0.05	0.026-0.057	0.045-0.095	0.112-0.181	0.048-0.086	0.083-0.128	0.066-0.128	0.026-0.068	0.054-0.094	0.026-0.074	0.034-0.066	0.257-0.327	0.046-0.088	0.027-0.063	0.107-0.149	0.131-0.182	0.116-0.176
Adergoole & Massbrook	0.021	0.011	0.048	0.018		0.039-0.074	0.104-0.156	0.038-0.076	0.115-0.182	0.019-0.047	0.013-0.037	0.022-0.066	0.1-0.167	0.042-0.08	0.049-0.104	0.059-0.109	0.031-0.07	0.05-0.08	0.015-0.055	0.037-0.064						



**Table 5.** Twenty six baseline river populations (Reporting units) identified from the hierarchical STRUCTURE analysis. The summary results (in %), of correct self-assignment (ONCOR) to these populations (diagonal values), including mis-assigned individuals are also shown. The darker colour in the green heat-map scale indicates stronger assignment levels.

Baseline River Populations / Reporting units	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1- Upper Deel River	52%	7%	-	2%	17%	4%	-	-	-	2%	2%	-	-	-	-	-	-	2%	4%	6%	-	-	4%	-	-	-	-
2- Deel, Brandra & Fortland Rivers	3%	57%	5%	-	14%	-	-	-	-	5%	-	-	-	-	-	-	2%	-	2%	3%	3%	-	3%	2%	-	-	-
3- Fiddaunglass River	1%	4%	73%	5%	14%	-	-	1%	-	1%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4- Castlehill River	4%	4%	-	56%	24%	2%	-	-	-	4%	-	-	-	-	-	2%	-	-	2%	4%	-	-	-	-	-	-	-
5- Addergoole & Massbrook Rivers	5%	11%	-	5%	62%	1%	-	1%	-	3%	3%	1%	-	-	-	-	2%	-	-	2%	3%	-	-	-	-	1%	-
6- Tobergall River	-	-	-	-	3%	72%	-	-	-	8%	8%	-	-	-	-	-	-	-	-	-	6%	-	-	-	3%	-	-
7- Clydagh River	-	-	-	1%	-	-	87%	1%	-	4%	1%	-	3%	-	-	-	-	-	-	-	1%	1%	-	-	-	-	-
8- Castlebar & Lower Manulla Rivers	4%	2%	-	-	2%	2%	11%	55%	-	2%	4%	2%	-	-	-	-	2%	4%	2%	5%	4%	-	2%	-	-	-	-
9- Upper Manulla River	-	-	-	-	-	-	-	-	93%	-	-	-	-	-	-	-	-	7%	-	-	-	-	-	-	-	-	-
10- Straide & Clonlea Rivers	2%	4%	-	4%	4%	4%	-	-	-	47%	6%	2%	-	-	-	-	2%	2%	9%	15%	-	-	-	-	-	-	-
11- Killeen & Spaddagh Rivers	-	9%	-	-	4%	-	-	6%	-	4%	61%	-	-	2%	-	-	2%	2%	6%	4%	2%	-	-	-	-	-	-
12- Pollagh River	4%	-	-	-	4%	-	-	4%	-	4%	68%	-	8%	-	-	-	-	-	-	-	8%	-	-	-	-	-	-
13- Yellow River	-	-	-	-	-	3%	-	3%	-	-	-	3%	90%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14- Glore River	2%	2%	-	-	-	-	-	-	-	2%	4%	-	87%	-	-	-	-	-	-	-	-	-	-	2%	-	-	-
15- Trimogue River	-	-	-	5%	-	-	-	-	-	5%	-	-	-	5%	84%	-	-	-	-	-	-	-	-	-	-	-	-
16- Swinford River	3%	-	-	3%	-	-	-	-	-	3%	-	-	-	-	-	-	73%	3%	3%	8%	3%	3%	-	-	-	-	-
17- Sonnagh River	-	2%	-	2%	-	-	-	5%	-	7%	5%	-	-	-	-	-	65%	-	-	9%	2%	-	2%	-	-	-	-
18- Mullaghanoe River	2%	2%	2%	-	-	-	-	2%	-	4%	5%	2%	-	-	-	-	-	73%	4%	5%	-	-	-	-	-	-	-
19- Owengarve River	9%	3%	-	-	3%	-	3%	-	-	12%	3%	-	-	-	-	-	-	-	44%	3%	-	-	-	21%	-	-	-
20- Einagh River	-	4%	-	-	-	-	-	3%	-	7%	9%	1%	-	-	-	-	3%	-	1%	70%	1%	-	-	-	-	-	-
21- Upper Owenaher	1%	3%	-	-	1%	1%	1%	1%	-	4%	-	-	-	-	-	-	-	-	4%	1%	78%	1%	1%	1%	-	-	-
22- Owenaher (lower), Mad & Upper Moy	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%	-	-	-	-	-	-
23- Yellow River (Foxford)	3%	3%	-	-	3%	3%	-	-	-	15%	-	-	-	3%	-	-	-	-	5%	-	3%	-	64%	-	-	-	-
24- Brusna (Bunree/Glenree) River	-	-	-	1%	-	-	1%	-	-	-	1%	-	-	-	-	-	-	-	-	-	-	-	-	96%	-	-	-
25- Easkey River	-	-	-	-	-	-	-	-	-	2%	-	-	-	-	-	-	-	-	1%	-	-	-	-	1%	95%	-	-
26- Palmerstown River	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4%	-	-	-	-	-	-	96%	-
27- Farm Fish (Roscrea & Leven)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100%

The results of the assignment for both adult lake trout (from Conn and Cullin) and sea trout (from Moy Estuary) to STRUCTURE-inferred reporting group/populations are summarised in Table 7 and also in Figure 12 for Lough Conn. Overall, ~79% (N = 384) of the adult lake trout and sea trout assigned to one of the identified baseline or reporting groups with high level of confidence ( $P \geq 0.7$ ). For fish caught in the lakes, there was generally a strong correlation between the broader area where the adult fish were captured and their assigned reporting group/population. Thus, 92% (N = 211) of the adult fish caught in Lough Conn assigned to a reporting group/population from the Western River Moy that directly flow into Lough Conn (Table 7A and Figure 12). The reporting group that includes the Addergoole and Massbrook rivers contributes the most at 42.4%. This is followed by the Deel, Brandra, and Fortland rivers reporting group at 28.4%, the Castlehill River at 14%, and the Upper Deel at 7.4%. The remaining 7% of the assignments were distributed among several locations: Tobergall in the Western Moy at 2.6%, the Brusna River in North Moy at 1.3%, and in the Eastern Moy, the reporting groups of Clonea & Straide at 1.7%, Killeen & Spaddagh at 0.9%, along with the Einagh River at 0.4% and the Owengarve River at 0.9%. There were no observed biases in the distribution of adult fish throughout the lake, indicating that adult fish from any population or reporting group appear to evenly disperse across the lake. Interestingly, the area of a river sub-catchment showed no correlation with its contribution (i.e. population or reporting group) to the lake brown trout stock. For instance, although the Addergoole & Massbrook sub-catchment wetted area is almost eight times smaller than that of the River Deel sub-catchment, the reporting group linked to this area still contributes a marginally larger share to the lake's brown trout stock (Table 8).



**Table 6.** Summary results, both in absolute numbers (A) and percentages (B), of individual assignment (ONCOR) of river caught adults to reporting groups/populations. Grey shaded cells in the table indicate agreement between river of capture and regional reporting group.

**A)**

River/Reporting Group	Upper Deel	Deel, Brandra & Fortland	Fiddaunglass	Castlehill	Adergoole & Massbrook	Tobergall	Clydagh	Castlebar & Manulla	Upper Manulla	Clonlea & Straide	Pollagh	Yellow	Glore	Trimogue	Killeen & Spaddagh	Swinford	Yellow (Foxford)	Sonnagh	Mullaghanoe	Einagh	Owengarve	Owenaher	Owenaher, Mad & Upper Moy	Brusna	Easkey	Palmerstown	Farm	Total	
Upper Deel	12																											17	
Brandra	3	5																											5
Fortland	1	1																				1							2
Fiddaunglass			16	1	1																	1							18
Castlehill				1																									1
Adergoole	1	3			4					1																			9
Massbrook					2																								2
Tobergall					1	2																							3
Clydagh							18			1																			19
Castlebar								19																					19
Manulla						1		7														1					1		21
Upper Manulla									8																				9
Clonlea										3																	1		8
Straide											17											1	1						6
Pollagh												7																	19
Yellow													6																7
Glore														18															6
Trimogue					1										2														19
Killeen																7													3
Spaddagh								1	1							18						2							11
Swinford															1							1							20
Yellow_Foxford				1						1							12												16
Sonnagh																		12											12
Mullaghanoe																			18										18
Einagh																				17									17
Owengarve																					7	1							8
Owenaher																						3	1						3
Owenaher, Mad & Upper Moy																						1	3	1					6
Upper Moy					1																						1		20
Mad																													2
Bunree																													4
Easkey																													4
Palmerstown																													15
Total	13	12	16	3	11	3	18	27	8	24	8	6	19	3	26	12	12	19	19	8	12	3	23	4	15	8	2	334	

**B)**

River/Reporting Group	Upper Deel	Deel, Brandra & Fortland	Fiddaunglass	Castlehill	Adergoole & Massbrook	Tobergall	Clydagh	Castlebar & Manulla	Upper Manulla	Clonlea & Straide	Pollagh	Yellow	Glore	Trimogue	Killeen & Spaddagh	Swinford	Yellow (Foxford)	Sonnagh	Mullaghanoe	Einagh	Owengarve	Owenaher	Owenaher, Mad & Upper Moy	Brusna	Easkey	Palmerstown	Farm	
Upper Deel	71	18																										
Brandra	100	50																										
Fortland	50																											
Fiddaunglass			89	6	6																							
Castlehill				100																								
Adergoole	11	33			44					11																		
Massbrook					100																							
Tobergall						67																						
Clydagh							95																					
Castlebar								90																				
Manulla									78																			
Upper Manulla										100																		
Clonlea											50																	
Straide												89																
Pollagh													100															
Yellow														5														
Glore															95													
Trimogue																67												
Killeen																	64											
Spaddagh								9	9									90										
Swinford																												
Yellow_Foxford																												
Sonnagh																												
Mullaghanoe																												
Einagh																												
Owengarve																												
Owenaher																												
Owenaher, Mad & Upper Moy																												
Upper Moy																												
Mad																												
Brusna																												
Easkey																												
Palmerstown																												
Farm																												

**Table 7.** Summary of results for individual assignment (ONCOR  $P \geq 0.7$ ) of adult fish captured in Lough Conn (A), Lough Cullin (B), and the Moy Estuary (C), presented in both absolute numbers and percentages, categorised by reporting groups or populations. Numbers and percentages are related to adult fish caught in each one of the target areas, respectively. The deeper hue on the green heat-map scale indicates higher assignment levels.

A) Lough Conn				
Assignment	Region	N	%	
Addergoole & Massbrook	Western Moy	97	42.4%	
Deel, Brandra & Fortland	Western Moy	65	28.4%	
Castlehill	Western Moy	32	14.0%	
Upper Deel	Western Moy	17	7.4%	
Tobergal	Western Moy	6	2.6%	
Bunree/Glenree (Brusna)	North Moy	3	1.3%	
Einagh	Eastern Moy	1	0.4%	
Clonlea & Straide	Eastern Moy	4	1.7%	
Owengarge	Eastern Moy	2	0.9%	
Killeen & Spaddagh	Eastern Moy	2	0.9%	
Total ( $P > 0.7$ )		229		

B) Lough Cullen				
Assignment	Region	N	%	
Addergoole & Massbrook	Western Moy	18	21.7%	
Deel, Brandra & Fortland	Western Moy	7	8.4%	
Castlehill	Western Moy	7	8.4%	
Upper Deel	Western Moy	4	4.8%	
Castlebar & Manulla	Western Moy	2	2.4%	
Tobergal	Western Moy	22	26.5%	
Clydagh	Western Moy	1	1.2%	
Bunree/Glenree (Brusna)	North Moy	2	2.4%	
Clonlea & Straide	Eastern Moy	5	6.0%	
Owenaher, Mad & Upper Moy	Eastern Moy	3	3.6%	
Einagh	Eastern Moy	3	3.6%	
Killeen & Spaddagh	Eastern Moy	3	3.6%	
Owengarve	Eastern Moy	5	6.0%	
Swinford	Eastern Moy	1	1.2%	
Total ( $P > 0.7$ )		83		

C) Moy Estuary				
Assignment	Region	N	%	
Bunree/Glenree (Brusna)	North Moy	66	91.7%	
Palmerstown	-	3	4.2%	
Addergoole & Massbrook	Western Moy	1	1.4%	
Castlehill	Western Moy	1	1.4%	
Tobergal	Western Moy	1	1.4%	
Total ( $P > 0.7$ )		72		

The adult fish assignment patterns for Lough Cullin (Table 7B) were similar to that observed in Lough Conn. Of all the adult fish caught in Lough Cullin, 77% (N = 64) are attributed to rivers in the Western Moy Region. The Tobergal River, which flows directly into Lough Cullin, was found to be the main source of the adult mixed stock in the lake, contributing 26.5%. Interestingly, a considerable proportion of fish caught in the lake can be traced back to the Addergoole & Massbrook reporting group, which represents ~22% and flows into Lough Conn. Lough Cullin adult fish assignments also includes the Deel, Brandra, and Fortland at 8.4%, Castlehill at 8.4%, Upper Deel at 4.8%, Castlebar & Manulla at 2.4%, and Clydagh at 1.2%. The remaining 26% is shared among seven reporting groups or

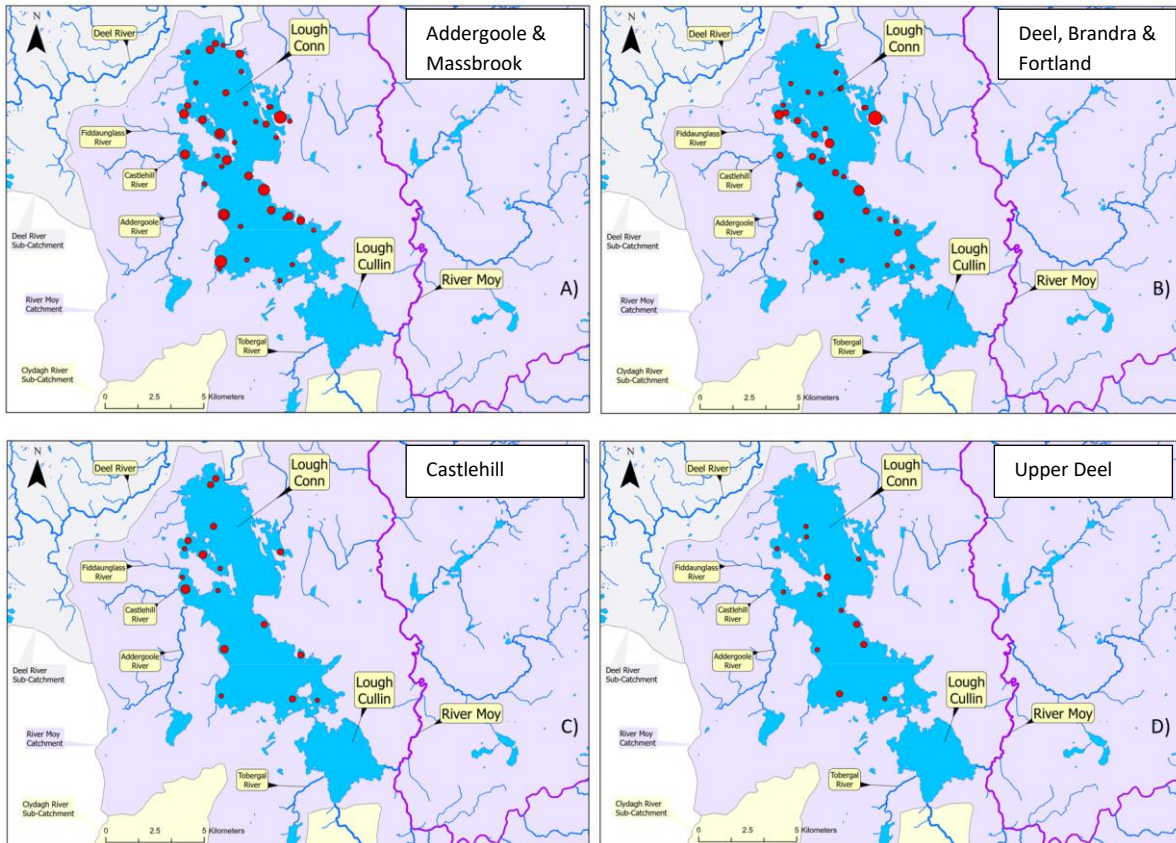
populations from the Eastern Moy Region. These include Clonlea & Straide at 6%, Killeen & Spaddagh at 3.6%, and Owenaher, Mad & Upper Moy at 3.6%, Einagh at 3.6%, Owengarve at 6%, and Swinford at 1.2%, along with Brusna at 2.4% in the North Moy. It is interesting to note that the comparatively few fish from Eastern Moy that reach the loughs typically remain in Lough Cullin, with very few travelling into Lough Conn. As observed with Lough Conn, there was no correlation between the wetted area of a river sub-catchment and the percentage contribution of its corresponding population or reporting group to the lake brown trout stock (Table 8). Consistent with the observations from river-caught trout, the relatively small number of adults caught in Lough Conn and Lough Cullin assigning with lower confidence (i.e.  $P < 0.7$ ), consistently aligned with their broader area of capture. This means that, regardless of the lower confidence level in their assignment, these fish consistently associated with a reporting group/population linked to the loughs they were caught. This indicates that these assignments, despite their lower confidence levels, are likely to be accurate. None of the adults found in Lough Conn and Lough Cullin were linked to the Farm baseline.

**Table 8.** River wetted area and % contribution of associated populations or regional groups to the Lough Conn and Lough Cullin adult lake stock.

<b>Lough Conn</b>				
<b>River sub-catchment</b>	<b>wetted area (km<sup>2</sup>)</b>	<b>as % of Moy catchment</b>	<b>as % of L. Conn catchment</b>	<b>GSI % contribution to lake</b>
Addergoole & Massbrook South	0.171	1.71	10.43	42.4
Deel	1.33	13.27	81.10	35.8
Castlehill	0.055	0.55	3.35	14
<b>Lough Cullin</b>				
<b>River sub-catchment</b>	<b>wetted area (km<sup>2</sup>)</b>	<b>as % of Moy catchment</b>	<b>as % of L. Cullin catchment</b>	<b>GSI % contribution to lake</b>
Tobergal	0.097	0.97	5.42	26.5
Clydagh	0.657	6.56	36.70	2.4
Castlebar	0.43	4.29	24.02	1.2
Addergoole & Massbrook South	0.171	1.71		21.7
Deel	1.33	13.27		13.2
Castlehill	0.055	0.55		8.4
<b>Eastern Main Channel Tributaries</b>				<b>24</b>

Most of the adult sea trout caught in the Moy Estuary mainly came from the Brusna system in North Moy, accounting for roughly 92% (N=66) of the sample. Palmerstown contributed a lesser amount, making up 4.2% of the total. Additionally, three other groups from Western Moy - Addergoole & Massbrook, Tobergal, and Castlehill - each made up 1.4% of the adult estuary sea trout population.

A significant finding of this study was that although 24 distinct Moy catchment genetic groups (populations and/or reporting groups) were identified, only about 54% (N = 14), mainly from the Western Moy region, seem to contribute to the adult brown trout stock in Lough Conn, Lough Cullin, and the Moy Estuary. This implies that brown trout linked to populations and/or reporting groups from the Eastern Moy region tend to stay in their native tributaries throughout their lives. Thus, they are not found in the adult samples from Loughs Conn, Cullin and estuary, nor in the River Moy's main channel, which is not a typical habitat for adult brown trout.



**Figure 12.** Individual assignment of adult fish caught in Lough Conn taking into consideration the specific location of capture. Assignments refer to populations and/or reporting groups as follows: A) Addergoole & Massbrook; B) Deel, Brandra & Fortland; C) Castlehill and D) Upper Deel. In each case, pie size is proportional to number of individuals (ranging from 1 to 9 avg. ~2). Reporting regions and/or populations with contribution less than 5% are not shown.

## 5. Summary & Concluding Remarks

The Moy catchment is a complex network of biological and genetic diversity. Historically, it has been shaped and altered by a variety of human based activities—ranging from arterial drainage and agricultural practices to urban development. Since the early 1900s, the catchment has been facing challenges associated with water quality, the construction of weirs, and the effects of hatchery and fish farm stocking. In addition, the introduction of non-native species such as roach and zebra mussel, along with the pressures from fishing, has further exacerbated its ecological imbalance. The main aim of this study was to understand the genetic diversity of brown trout within the catchment, identifying the rivers and tributaries that are essential to sustaining the adult trout populations of Loughs Conn & Cullin as well as the main Moy channel. The study also aimed to address several more specific questions outlined in Chapter 2, Section 2.2.

### **Moy Catchment contemporary population structure (Objective 1)**

Despite the several environmental challenges outlined above, that are known to negatively impact population substructuring, genetic diversity and consequently the long-term survival of populations, results from this comprehensive genetic study reveals a significant degree of natural resilience among the wild brown trout populations in the catchment. Thus, the brown trout within the Moy catchment are differentiated into 24 genetically distinct populations distributed across the western (N=9), eastern (N=14) and northern (N=1) regions of the Moy catchment. Two additional genetically distinct populations were identified within the Easkey and Palmerston catchments, respectively.

In line with findings from other similar studies carried out in Ireland and elsewhere (e.g. Prodöhl *et al.*, 2019; Delanty *et al.*, 2020, 2022; Hynes *et al.*, 2022), the patterns and levels of genetic variation observed among the brown populations in the Moy catchment are likely the outcome of isolated divergence, influenced by both Ireland's recent post-glacial history and changes to the environment brought about by human activities, particularly since the early 1900s onwards. The contemporary pattern of population structuring within the Moy catchment, that is the western, eastern and northern groupings, is likely explained by the physical landscape of the system, especially the presence of Lough Conn and Lough Cullin.

A significant finding of this investigation was the identification of brown trout populations exhibiting clearly distinct life history strategies. Brown trout is characterised by two resident (river-resident and lake-resident), and three main facultative migratory life histories: 1) movement downstream and upstream within a river system (fluvial-adfluvial potamodromous), 2) migration between a river and a lake (lacustrine-adfluvial for inlets or allacustrine for outlets, potamodromous), and 3) migration between freshwater and the sea (anadromous). The choice between remaining river-resident and migrating is a balance between increased feeding opportunities and subsequent growth benefits of moving to a specific habitat against the potentially higher risks of mortality and the expenditure of energy associated with migration (see Ferguson *et al.*, 2019 for a comprehensive review). Within the Moy catchment, populations from the western region generally exhibit a lacustrine-adfluvial (inlet) life history, whereas eastern populations display river residency, fluvial-adfluvial, and also lacustrine-adfluvial (outlet) life histories, with some variations in each case including anadromy. The sole population in the northern part of the catchment typifies the anadromous life history strategy. Given that lake residency cannot be entirely dismissed, it's possible that the Moy catchment encompasses

all known life history strategies in brown trout. These varied strategies have likely developed as adaptations to the Moy catchment's physical landscape.

### **Quantification of the relative contribution of identified populations to the adult brown trout fishery to Loughs Conn and Cullin (Genetic Stock Identification) (Objective 2).**

Nearly 90.5% of adult lake trout caught in Lough Conn and Cullin, as well as sea trout from the Moy Estuary, were confidently linked to the baseline populations or reporting groups identified in this study. Some 92% of adult fish caught in Lough Conn, were associated with populations or reporting groups from the Western River Moy. Brown trout from the Addergoole and Massbrook rivers are the leading contributors (42%), despite their smaller catchment size compared to the River Deel (39%), challenging the assumption that larger catchment areas contribute more significantly. This pattern also holds for Lough Cullin, where 77% of trout were also attributed to the Western Moy Region, with notable contributions from rivers flowing into both Lough Conn (21.7 % Addergoole and Massbrook rivers) and Lough Cullin (26.5% Tobergal). The sea trout in the Moy Estuary primarily originated from the Brusna system (91.7%). An important finding of this investigation is that the predominant contributors to the adult trout stocks in Loughs Conn and Cullin are the identified populations or reporting groups primarily from the Western Moy. Thus, brown trout from populations or reporting groups identified in Eastern Moy trout tend to remain within their natal rivers/tributaries throughout their lifecycle. This highlights the non-uniform dispersal of brown trout displaying distinct life history strategies across their physical landscape and emphasises the lack of a direct correlation between the area of a river sub-catchment and its contribution to the adult lake trout caught in the lakes. In contrast to the findings reported here, other research on brown trout population genetics in Ireland (e.g. Delanty *et al.*, 2021, 2022), indicates a definite positive correlation between the wetted area of a catchment and its contribution to the adult brown trout stock in lakes. Therefore, it is important to avoid generalising and to evaluate each situation individually.

### **Assessment of the potential impact(s) of the stocking history involving farmed derived fish on the wild brown trout populations inhabiting the broader River Moy catchment (Objective 3).**

Historical records indicate limited instances of brown trout stocking in the Moy catchment area, with the majority of these events associated with the Castlebar lakes (Bilberry & Islandeady), where approximately 1.2 million fish of farm origin are reported to have been introduced (IFT annual report 1962 – 1980). Similar to what was observed in other brown trout genetic based studies in Ireland (Delanty *et al.*, 2021 & 2022, Hynes *et al.*, 2022), there was no evidence indicating any significant impact(s) resulting from this stocking history. Brown trout originating from the Roscrea fish farm possess a unique genetic makeup that are very distinct from wild brown trout, making them easily identifiable. The fact that, apart from a few exceptions noted below, they were not found in this comprehensive survey, confirms what has been reported in previous studies (Delanty *et al.*, 2021 & 2022, Hynes *et al.*, 2022). When introduced into the wild as fry or juveniles, fish from hatcheries exhibit significantly lower survival rates compared to their wild counterparts. Ferguson (2007) outlines multiple factors contributing to the frequently observed poor outcomes of fish stocking efforts, such as: the quantity of stocked fish in relation to the native populations; diminished survival rates of hatchery fish in the wild owing to disrupted natural selection processes; methods of transport and stocking; prevailing environmental conditions; physiological and morphological disparities; differences in feeding and predator-evasion tactics; and increased vulnerability to fishing. In summary,

the impact of such stocking practices, especially if not sustained over time, is minimal. An interesting exception was found in the Owenaher River, above an impassable waterfall, where evidence of farmed trout origin was detected. Although no recent records of stocking exist for this river, it is possible that unrecorded introductions may have occurred in the past. In situations where wild brown trout are absent, such as waters above impassable waterfalls, it is possible that stocked fish of farm origin might survive and thrive given suitable conditions, including ample foraging, shelter and breeding habitats. This phenomenon has been reported in other studies where trout populations were wiped out and later successfully reintroduced (Prodöhl *et al.*, 2019). Should this be the case, the Owenaher brown trout situated above the waterfall might constitute a feral population. Further research is required to verify this hypothesis. It is important to recognise that although there was no substantial evidence of genetic mixing between farm and wild brown trout, the presence of farm-bred trout in the wild can still adversely affect the native fish, especially regarding competition for food resources. Despite farm fish having lower fitness and survival rates compared to wild trout, their competition for food and shelter can add extra pressure on the survival of the wild population (see Ferguson 2007 for further information).

**Assessment of the impact of barriers (natural and manmade) to fish migration and their potential impact(s) on contemporary population genetic structure (Objective 4).**

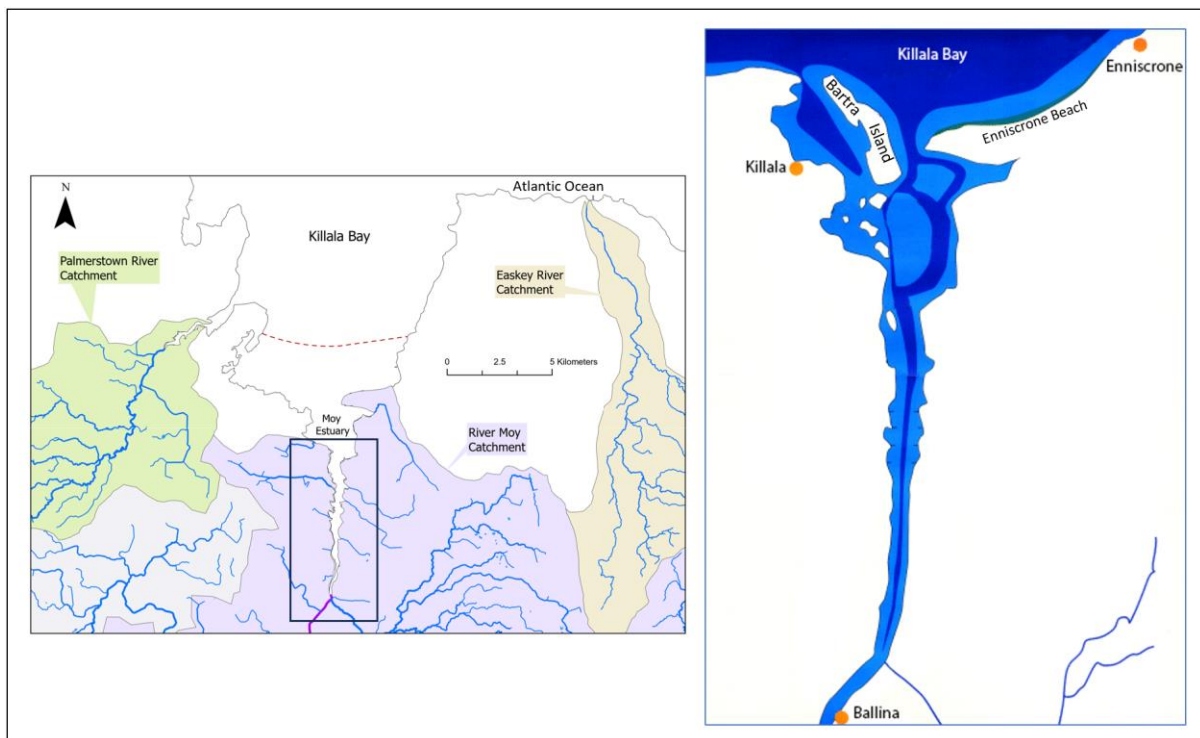
In the Moy catchment system, with limited exceptions, there were no significant indications of barriers inhibiting the migration of brown trout. An exception was noted in the upper Deel River, where the brown trout populations upstream and downstream of a natural waterfall are genetically distinct, with evidence suggesting gene flow predominantly from upstream to downstream. Another exception was in the upper Clydagh River, where again distinct genetic differences were observed between populations separated by a natural waterfall. Enhancement efforts (part of the TAM enhancement) have been made to improve salmonid migration in this river, with partial success indicated by evidence of gene flow in both directions across the waterfall. A third exception occurred in the Owenaher River, where the populations above and below the waterfall are also genetically distinct. The upstream population, as mentioned earlier, seems to have originated from historical stocking with farm-bred brown trout.

**To identify the potential source populations within the broader River Moy catchment and adjacent catchments, as well as their contribution to the anadromous brown trout (sea trout) stock that forage in the Moy Estuary. (Objective 5).**

Contrary to previous assumptions that sea trout from the Palmerstown and Easkey catchment might use the Moy Estuary for feeding, the findings of this study have demonstrated otherwise. The vast majority of adult sea trout captured in the Moy Estuary were genetically identified as originating from the Moy, predominantly from the Bunree River, accounting for 91.7% of the samples. A minor contribution comes from the Palmerstown catchment at 4.22%, while no adult sea trout, provided to this study, from the Moy Estuary were linked to the Easkey catchment. It is important to note, however, that the Palmerstown and Easkey Rivers do not flow into the Moy Estuary directly (Fig. 13). The Palmerstown River empties into Killala Bay, whereas the Easkey River meets the North Atlantic Ocean directly. Therefore, it is possible that sea trout from those catchments are not necessarily sharing the same feeding grounds as Moy derived sea trout. The prior thought was that Moy derived sea trout might spawn across various rivers in the catchment, not limited to the lower tributaries



(Bunree River). Thus, observations by IFI staff have noted adult brown trout, resembling sea trout, in the Moy's upper main stem and some mid-system tributaries (such as the Foxford Yellow River) as well as in Loughs Conn and Cullin. Nevertheless, the results of the current study do not corroborate these observations. In the samples analysed, a very small number of adult sea trout from the Moy estuary were attributed to populations outside of the Bunree system (Addergoole & Massbrook, Castlehill and Tobergal). These results, however, must be interpreted with caution due to the non-randomised sampling method employed for the adult sea trout from the estuary. To obtain a comprehensive understanding of the contributions from other Moy tributaries to the adult sea trout population in the Moy Estuary and Killala Bay, further research is needed, incorporating a temporal aspect and an expanded sampling region within the Moy estuary. In many other large Irish catchments (Boyne, Slaney, Suir, Cork Blackwater, and Killarney) returning sea trout tend to spawn in tributaries close to tidal areas rather than traveling far up the main river. The results presented in here are in agreement with this spawning behaviour. Angling records indicate an annual capture of approximately 100 adult sea trout within the Moy catchment (unpublished data from IFI Ballina).



**Figure 13.** The map illustrates the Moy Estuary, the source of the sea trout samples for this study. Both the Palmerstown and Easkey Rivers do not directly flow into the Moy Estuary. The Palmerstown River discharges into Killala Bay, while the Easkey River flows directly into the North Atlantic Ocean.

## **Acknowledgments**

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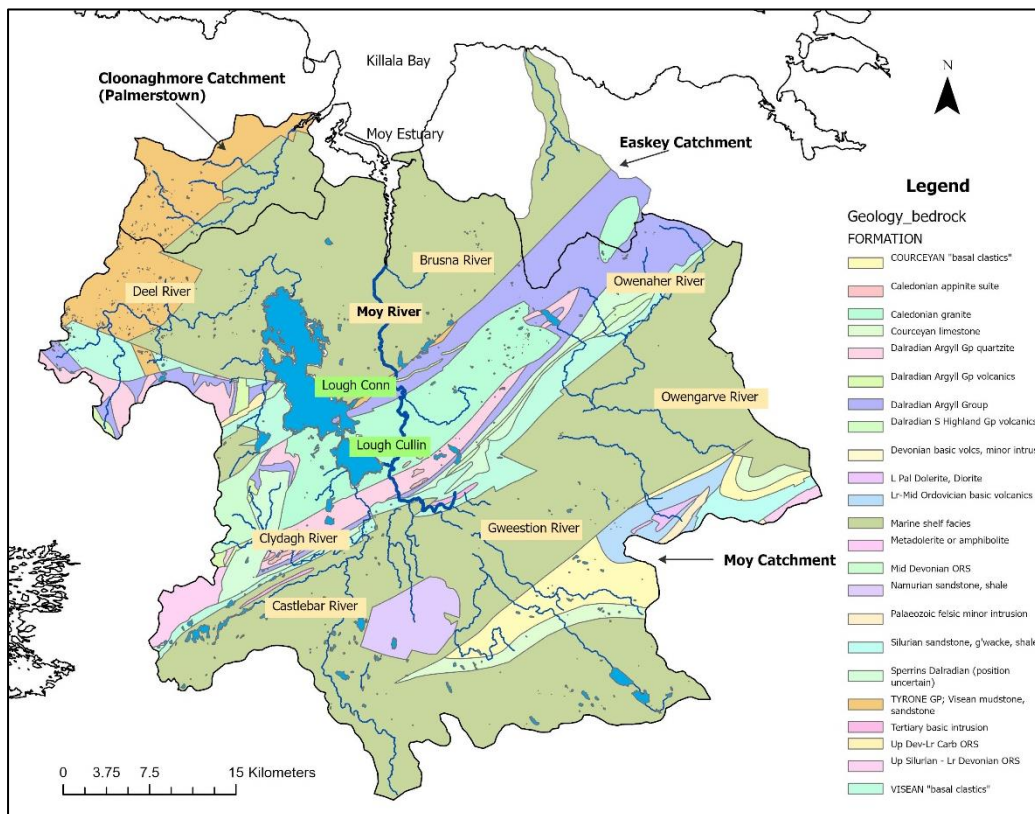
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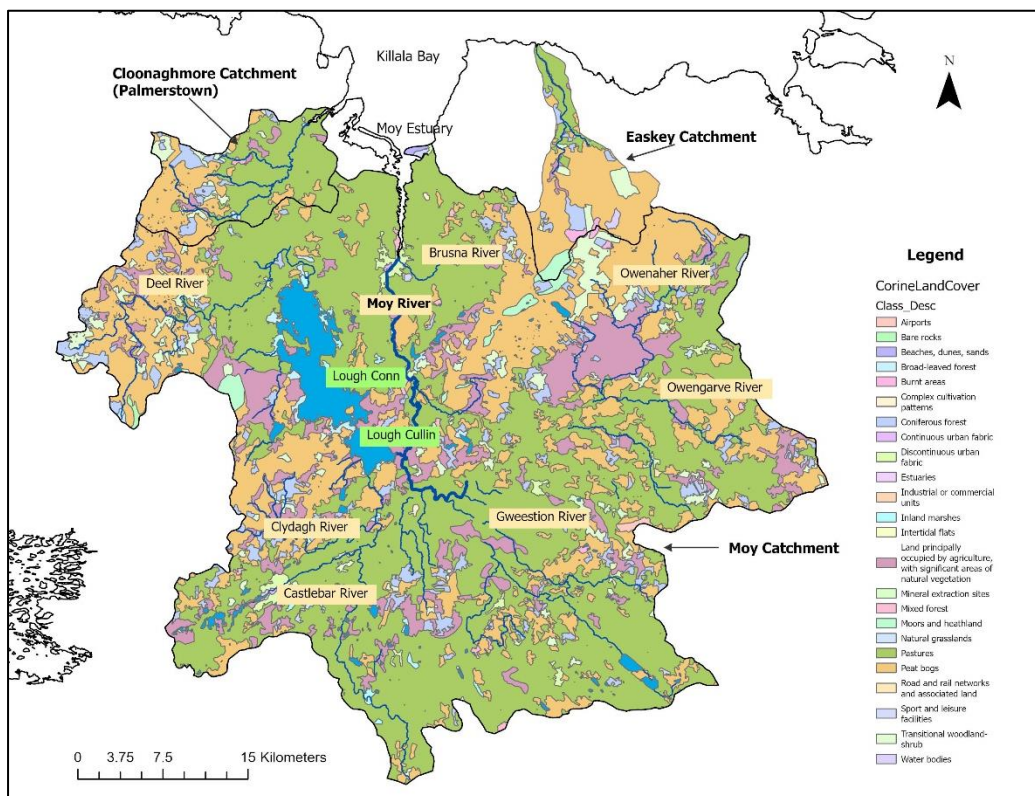
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## APPENDIX I: Geology and Bedrock (GSI, 2023)

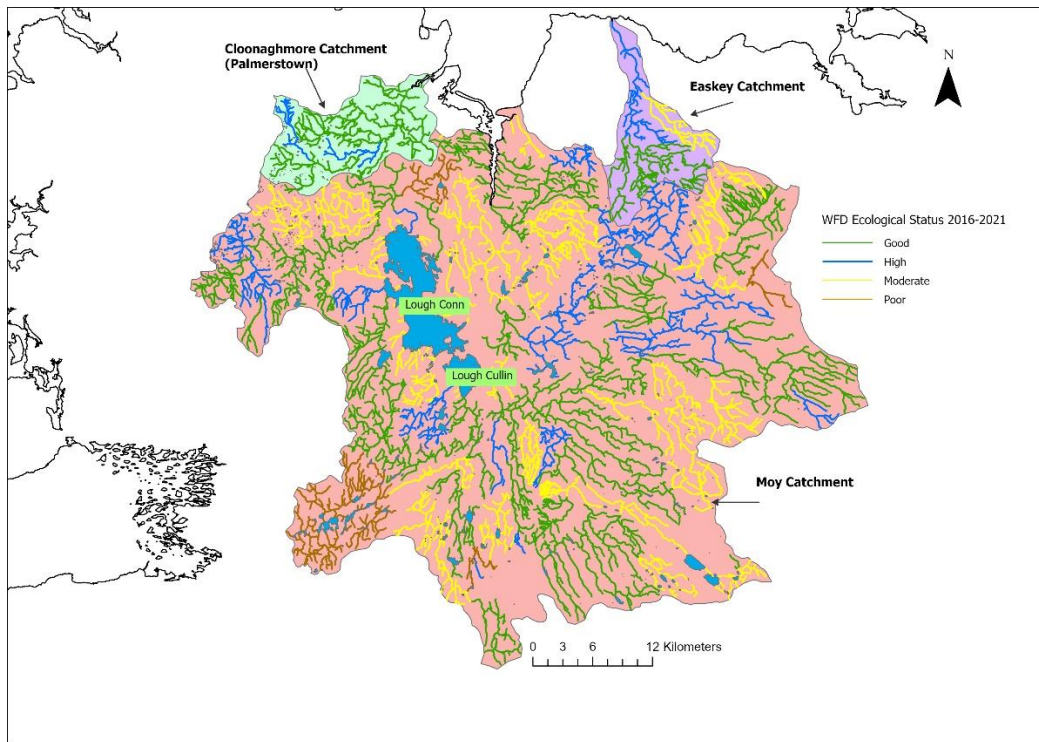


## APPENDIX II: CORINE Landcover (CORINE 2018)





**APPENDIX III: Water Framework Directive Status – River Status 2016-2021 reporting Period (EPA 2023)**



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

**[www.fisheriesireland.ie](http://www.fisheriesireland.ie)  
[info@fisheriesireland.ie](mailto:info@fisheriesireland.ie)**

**+353 1 8842 600**

