

Population Structure and Genetic Stock Identification of the Liffey Catchment Brown Trout

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1. Introduction

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

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

In 2014, Inland Fisheries Ireland (IFI) commissioned Queens University Belfast (QUB) to carry out a genetic study of the Liffey Catchment brown trout, in conjunction with two other Dublin river systems namely the Dodder and the Tolka, with the aim to investigate possible changes in the genetic make-up of populations as a consequence of a changing environment. The study is based on a large-scale biological survey of the Liffey main channel and its main tributary rivers and streams.

The project received monetary support from the many Liffey angling clubs – Clane Trout and Salmon Anglers Association (CTSAA), North Kildare Trout and Salmon Anglers Association (NKTSAA), Dublin Trout Anglers Association (DTAA), Ballymore Eustace Trout and Salmon Anglers Association (BMETSAA), Dublin City Council and Intel. The same angling clubs also provided adult trout scale samples to the study.

The results of the Liffey Catchment system are reported here.

2. Liffey Catchment Study Area

The River Liffey rises only 12 miles south of Dublin city (Kippure in the Wicklow mountains), but flows in a huge crescent meandering journey west, north and northeast through Wicklow and Kildare before turning east towards Dublin City. It flows for over 82 miles (120km) before entering the sea at Dublin Bay. It drains a catchment of nearly 530 square miles (1373km²). It starts as a poor acid mountain river and is transformed into a rich trout river as it glides and meanders through the plains of Co. Kildare (Figure 1). The River Liffey flows over a range of differing geologies, from granite to sandstone to sandstone-limestone and finally to pure limestone. Some of the fastest growing brown trout ever recorded in Ireland were observed in the Liffey main channel near Lucan (Kennedy & Fitzmaurice, 1971). The main land uses are peat bog and forestry in the upper catchment, agriculture in the middle reaches and urban as the river flows through Dublin suburbs and the city.

Along the course of the River Liffey there are three Electricity Supply Board (ESB) hydroelectric power stations, with associated dams and reservoirs, completed in 1943 (Golden Falls), 1944 (Poulaphouca) and 1949 (Leixlip) respectively. Water abstraction is also an ongoing pressure. The Poulaphouca and Leixlip Reservoirs supply most of the drinking water to Dublin City. Management at these stations means that water flows are highly controlled and volume discharge is regulated by the ESB. The river is therefore subject to

artificial spates that are not directly related to natural rainfall patterns. The dam at Poulaphouca was built on a waterfall that was naturally impassable to migratory fishes prior to dam construction, and thus salmon have been limited to the main channel and tributaries downstream of the original falls at Poulaphouca (Went, 1945-1948) (Fig. 1).

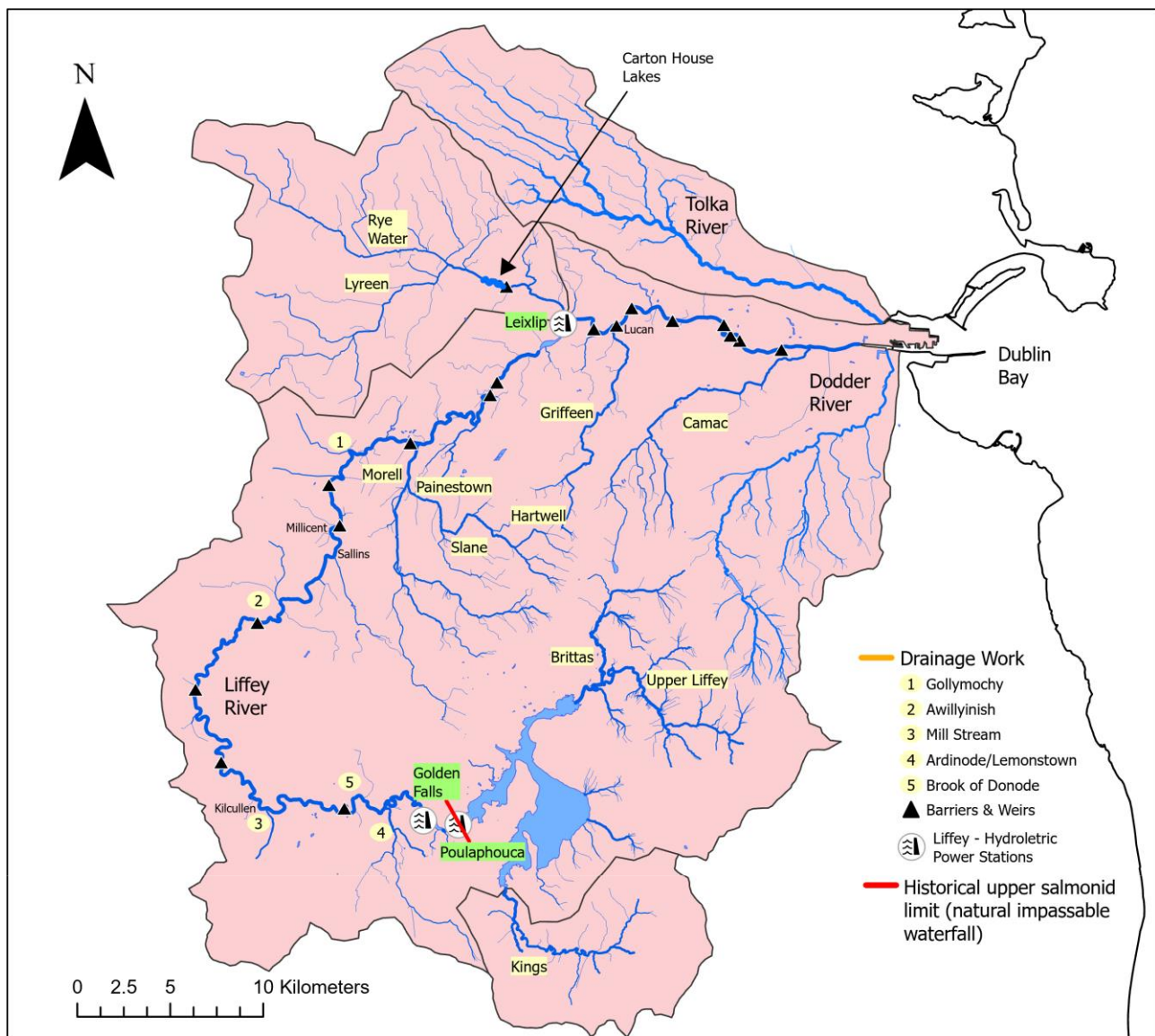


Figure 1. Area of study including main geographical features and hydroelectric power stations. ▲ indicate locations of weirs and barriers erected along the Liffey main channel.

Over the years more than 20 weirs and barriers have been erected along the Liffey main channel alone (Fig. 1). All these structures are likely to pose some level of obstruction to salmonids and other migratory fishes, including eels and lampreys, at different times of the year and depending on water levels. While many of these weirs are now redundant and several are in disrepair, in addition to disconnecting the river, they continue to impound water and contribute to sediment build up for extended lengths of the river. Movement of fish in both the upstream and downstream direction can be impeded especially during periods of low flow.

During the 1850's the Duke of Leinster initiated artificial drainage in the Rye Water catchment, by deepening the river and installing field drains (O'Reilly, 2002). Drainage schemes typically involve 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). It was also during this period that two artificial lakes were also created along the course of the river by building a waterfall & weir to hold back water thus creating the small lakes. Further arterial drainage work on the Rye Water and its tributary, the Lyreen, was subsequently carried by the Office of Public Works (OPW) over a two-year period from 1952 to 1954, to alleviate persistent flooding in Leixlip village. As part of this Rye Water Arterial Drainage Scheme OPW operate an annual maintenance programme across the sub-catchment. Separate to this, an OPW Flood Management Scheme (lead by Kildare County Council) has commenced in 2020 across the Morell sub-catchment (<https://www.morellfms.ie/>). The Flood Management Scheme will see work being carried out on the Morell main channel, Slane, Hartwell and Painestown (Kill) rivers. Some works have already been completed. As part of the Morell Flood Management Scheme works will include;

- construction or restoration of over 9,000 metres of sloped embankments
- construction of up to 480 metres of flood walls to direct the flood water away from high-risk areas
- realigning two streams, and
- up to 11 culvert alterations/upgrades

The wider River Liffey catchment has suffered from water quality issues historically, and this impact continues today. Sub-catchments currently most at risk include the Lyreen and Rye Water, the Griffeen and the Camac. While there have been improvements in several of the waste water treatment (WWT) plants located and discharging into the Liffey middle reaches, water quality continues to be a noted pressure on the Liffey system. The changes noted in Water Framework Directive (WFD) Ecological Status (2013-2018), since the previous Status report (2010-2015), suggest that there have been improvements in some areas (Rye Water tributaries, Griffeen and main channel lower) but a decline in others (Morell, main channel upper, Kings and upper Liffey tributaries) (Delanty & Shephard, 2021).

Supplemental stocking within the Liffey catchment has been carried out as a mitigation measure to counteract the potential negative impact of some of the catchment's issues, such as water quality and the hydroelectric schemes, on local brown trout populations. Initially, the source of brown trout for stocking was the IFI operated Roscrea Fish Farm (Co. Tipperary). During the period from late 1960's to 2014 the Liffey catchment was annually stocked out with farmed reared trout fry (0+), summerlings, yearlings and 2+ yr olds (IFT annual reports 1957 - 1987, IFI unpublished data 2000 - 2014). Over 2 million fish (of all life stages, ova to 2+ year old adults) have been stocked out across the Liffey system. The majority of which were unfed fry and 0+ fry (over 1.5 million) (IFI unpublished data). During a period between the mid 1980's to mid 1990's local angling clubs also ran a trout hatchery at Roseberry, Co. Kildare (NKTSAA) with the aim of supplemental stocking. This hatchery reared on trout ova, obtained from the Roscrea Fish Farm and/or secured Liffey trout broodstock, which were 'stripped' and fertilised ova developed and reared in the Roseberry hatchery. The fish were then stocked out into the Liffey main channel between Kilcullen and Sallins as yearlings. Currently only the Poulaphouca Reservoir and two small ponds, both of which have grids on their outflow, receive stocked farmed fish (2+ brown trout and rainbow trout).

Study Objectives

The main objectives of the project were:

- To assess the potential impact(s) of the stocking history in the Liffey on the wild brown trout populations
- To describe the contemporary brown trout population genetic structure of the River Liffey and its main sub-tributaries
- To quantify the relative contribution of identified populations to the adult brown trout fishery (Genetic Stock Identification).
- To assess the impact of barriers (natural and manmade) to fish migration and their potential impact(s) on contemporary population genetic structure
- To identify potential populations producing anadromous brown trout (i.e. sea trout) within the Liffey catchment system

3. Materials and Methods

Seven main River Liffey tributary rivers (Griffeen, Camac, Rye Water, Morell, Upper Liffey, Brittas and Kings), and associated sub-tributaries, were selected for sampling, along with five smaller tributaries (Gollymochy, Awillyinish, Mill Stream, Brook of Donode and Ardinode/Lemonstown) (Fig. 1). Rationale for river and/or tributary selection, including sampling sites, was based on information derived from previous electrofishing surveys, redd count surveys and habitat surveys, carried out over several years that were identified as key spawning and nursery grounds for brown trout (IFI unpublished data). Sampling details are provided below and summarised in Table 1. A total of 1535 specimens, collected between 2012 and 2015, were available for genetic analyses. Sampling details including fish length, weight, and capture location, for all samples, were recorded on an electronic database. Geographical locations for all sampling sites are shown in Fig. 2.

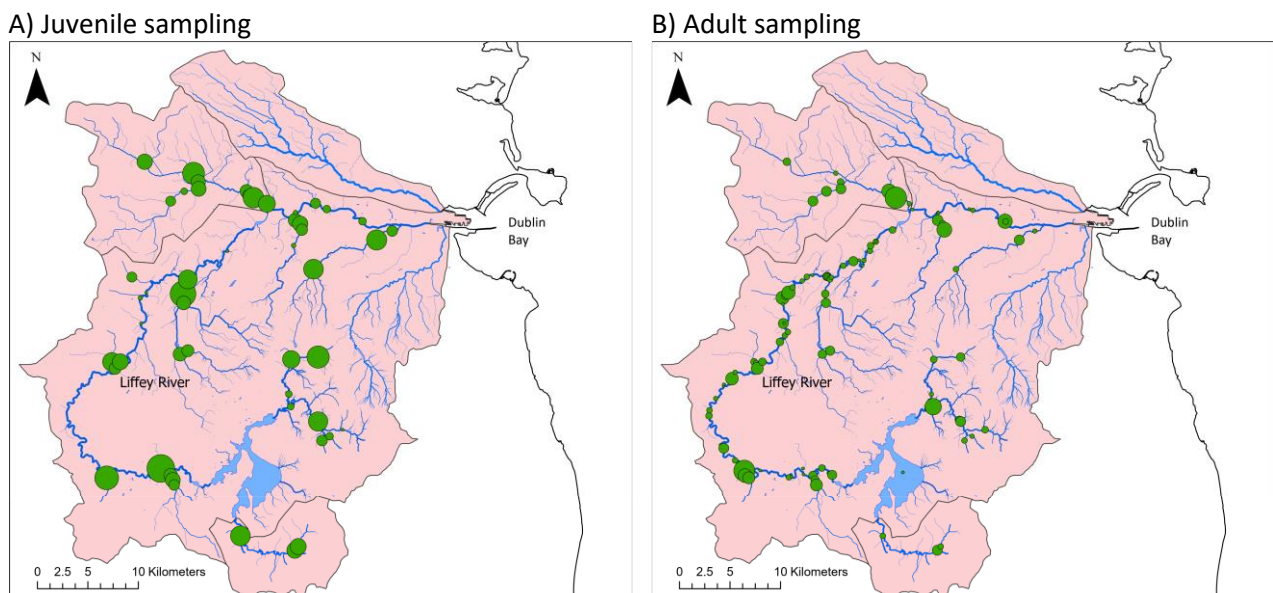


Figure 2. Locations of (A) juvenile and (B) adults sampling including number of individuals sampled per site

Sampling Details

Genetic Baseline

Non-destructive biopsy material (fish scales) for the identification of the baseline river samples were collected by electrofishing juvenile trout from nursery sections of the selected tributaries (Table 1/Fig. 2). Where possible, a minimum of three locations were sampled from within each sub catchment to ensure unbiased/representative sampling of putative populations, and to minimise sampling of siblings (family bias). In all instances, dry scales were stored in individual envelopes at room temperature prior to genetic analyses. A total of 893 juvenile trout were initially sampled for genetic analyses.

Genomic DNA from fish (N=131) of farm origin (Roscrea hatchery), available from previous brown trout-based population genetic studies carried by the IFI and QUB (Delanty *et al.* 2020, 2022) were also included in the analyses as the baseline reference for the Liffey stocked fish.

Adult Sampling

Adult trout caught in rivers by electrofishing, from sites across the catchment, were also non-destructively sampled for analyses. All fish larger than 16cm in length were classed as adults. The rationale for this length threshold is that fish above this size (>1+) have been observed moving (i.e. migrating) further afield from the locations they were sampled in. Thus, they are likely to represent transitory (migratory) adult fish and possibly do not belong to a putative local population. To minimise possible biases in the identification of baseline genetic populations, these individuals were treated as “unknown” adults. Scales from adult trout samples from the Liffey main channel, and a small number of other angling locations, were also provided by several Liffey angling clubs. A total of 642 adults were initially sampled for genetic analyses. Of these, ten individuals caught on the Lower Liffey main channel were phenotypically (i.e. visual morphology inspection) identified as sea trout (i.e. anadromous brown trout).

Table 1. Number of sites and brown trout individuals (juveniles and adults) sampled as part of this genetic study. *Samples collected* refer to the total number of individuals caught while *Samples Analysed* refers to samples for which genetic data was successfully generated for analyses.

	Number of sites	Samples Collected			Samples Analysed		
		Juveniles	Adults	Total	Juveniles	Adults	Total
<i>Liffey River</i>	50	893	642	1535	750	529	1279
Upper Liffey	9	50	76	126	47	54	101
Brittas	4	57	14	71	57	13	70
Kings	4	70	15	85	62	14	76
<i>Liffey tribs:</i>							
Ardinode/Lemonstown	3	44	24	68	39	23	62
Awillyinish	1	25	5	30	23	4	27
Brook of Donode	1	51	3	54	47	1	48
Gollymochy	1	9	0	9	8	0	8
Mill Stream	1	39	11	50	36	10	46
Morell	8	107	33	140	104	30	134
Rye Water	11	208	96	304	166	91	257
Camac	3	67	14	81	62	13	75
Griffeen	4	45	33	78	43	29	72
Liffey Main Channel		121	318	439	56	247	303

Genetic Analyses (DNA Profiling)

Genomic DNA from all brown trout specimens sampled for this study, was extracted using the Promega Wizard SV 96 genomic DNA purification kit 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 20 microsatellite loci (*Ssa85*, *One102a*, *One102b*, *One108*, CA054565, *Ssa416*, *One103*, *Cocl-Lav-4*, *One9ASC*, CA048828, CA053293, BG935488, *SsaD71*, *SaSaTAP2A*, MHCI, *Ssa410UOS*, *ppStr2*, *ppStr3*, CA060177 and *Ssa197*) developed and/or 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 statistical genetic analyses.

Statistical Analysis

Identification of Baseline populations

To ensure that only high quality data was used for analysis, following genetic screening (i.e. genotyping), all individual juvenile trout, which could not be unambiguously scored for at least 14 of the 20 microsatellite loci (70% of scored loci), were removed from the main data set (see *Samples Analysed* in Table 1). In order to ensure an unbiased sample for the identification of the baseline populations, all individuals caught in the same site were checked for the presence of individuals with family ties (i.e. full sibs) using the Colony v2.0.6.2 program. On instances where more than three individuals were identified as siblings, only three (randomly selected) were retained for analyses (Waples & Anderson, 2017).

Population genetic structuring within the baseline data were investigated using the Bayesian framework implemented in the programme STRUCTURE V2.4 (Pritchard *et al.*, 2000) following the hierarchical approach suggested by Rosenberg *et al.* (2002). This approach allows for the identification of major genetic groups within the data that are potentially related by common ancestry and, subsequent further partitioning these major groups down to populations (i.e. genetic baselines). To ensure an unbiased sample data set for the examination of Liffey brown trout population structuring (i.e. definition of the baseline river populations), adult fish (any individual over the fork length of 16 cm) were excluded from the STRUCTURE analysis. These “unknown” adults were later 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 (contemporary and hatchery/farm) in an initial analysis to examine the potential impact to the genetic composition of the Liffey River brown trout over time, resulting from the extensive stocking history. The hatchery sample (Roscrea) was used as the reference baseline for the identification of stocked fish Liffey samples. The hatchery/farm samples were removed from subsequent STRUCTURE analyse where the objective was to access patterns of contemporary population structuring.

Summary statistics (e.g. number of alleles per locus, allelic richness (AR), observed and expected heterozygosity, tests for conformity to HW expectations) were calculated for the baseline STRUCTURE inferred populations using the *divBasic* function from the *diveRsity* package (Keenan *et al.*, 2013). Pairwise F-statistics (both θ ; 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 by bootstrapping (1000) using *diveRstiy*.

Genetic Stock Identification

The power and usefulness of the STRUCTURE inferred population baseline for individual assignment (i.e. to assign an adult individual to the correct population baseline) was assessed with the self-assignment test implemented in the program ONCOR (Kalinowski *et al.*, 2007). This program was also used to estimate assignment accuracy to regional reporting groups (groups of geographically close and genetically similar baseline populations). Finally, ONCOR was used to assign adult river caught samples to putative inferred populations, with an “ad-hoc” assignment *P* value of ≥ 0.7 being considered as robust (i.e. biologically meaningful) assignments (Prodöhl *et al.* 2017).

4. Results

From the 1,555 brown trout (893 juveniles and 642 adults), caught from 50 tributary river sites and several locations along the Liffey main channel, good quality genetic data was generated for 1,279 (82%) specimens (750 [84%] juveniles and 529 [82%] adults). See Table 1 and Fig. 2.

The result of the hierarchical STRUCTURE analysis assessing the potential impact of stocking on the wild Liffey River brown trout is summarised Fig. 3. The genetic data is best explained by two groups with a clear separation between wild (Liffey River) and hatchery/farm derived brown trout. Notwithstanding the extensive stocking history within the Liffey River catchment, there is no major evidence suggesting substantial introgression of hatchery/farm derived genes into wild brown trout (i.e. mixing between the genetic make-up of wild versus hatchery fish). Minor evidence for introgression, however, was noted in the Griffeen River.



Figure 3. STRUCTURE plot illustrating differences in the genetic makeup between Liffey River and hatchery/farm derived brown trout. Individual brown trout are represented as thin vertical coloured lines. Different colours represent distinct genetic groups. Multi-coloured individual vertical lines are indicative of introgression between groups. Samples from different sources (wild versus hatchery/farm) are separated by a thin vertical white line.

Subsequent STRUCTURE analysis involving the Liffey samples only (i.e. excluding hatchery/farm brown trout) identified four brown trout groups as follows: 1) Upper Rye Water & Lyreen; 2) Liffey Above Dam; 3) Griffeen & Camac rivers, and 4) Liffey Mainstem (Fig 4 L0). Level 1 STRUCTURE analysis (Fig. 4 L1) of brown trout from the Upper Rye Water & Lyreen group further differentiated them into two distinct sub-groups associated with their location. Brown trout from the Liffey Above Dam group were resolved into 1) Upper Liffey & King’s River and 2) Brittas River; brown trout from the Griffeen & Camac groups resolved into two sub-groups, again linked to their sampling locations; and those comprising the Liffey Mainstem were further partitioned into

three sub-groups as follows: 1) Upper Liffey Mainstem; 2) Lower Liffey Mainstem and 3) Lower Rye Water. With one exception, no further substructuring was noted within the groups identified at Level 1. Brown trout from the L1 Upper Liffey & Kings sub-group, however, was again found to resolve into two distinct groups explained by their sampling location (Fig 4. L2).

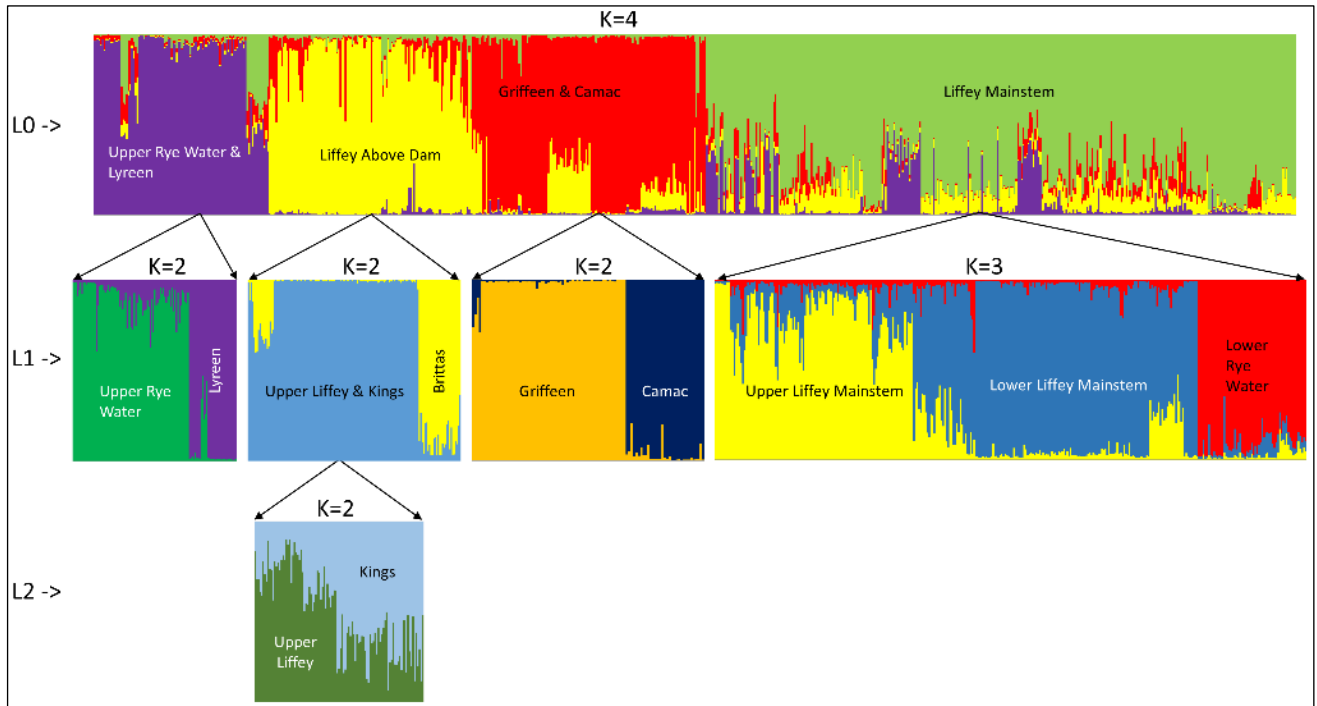


Figure 4. Hierarchical (three levels) STRUCTURE plots of brown trout from the Liffey River catchment illustrating contemporary population structuring. In each case the best number of genetic groups (K) explaining the data is shown. Different colours represent distinct genetic groups/populations. Multi-coloured individual vertical lines are indicative of genetic introgression between brown trout from the different groups.

The final result of the hierarchical STRUCTURE analyses provided evidence for the presence of ten brown trout populations within the Liffey River catchment. These populations were as follows; 1. Brittas River, 2. Upper Liffey River (above Poulaphouca dam), 3. King's River, 4. Upper Liffey Mainstem (area d/s Poulaphouca – Ballymore Eustace (BME) to Millicent Br.), 5. Lower Liffey Mainstem (d/s of Millicent Br. to Leixlip), 6. Lower Rye Water, 7. Upper Rye Water, 8. Lyreen, 9. Griffeen River and 10. Camac River. See Figure 5.

To further examine the genetic relationships among the inferred brown trout populations within the Liffey River 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), confirms population structuring identified in the STRUCTURE analysis and, clearly indicates a strong overall relationship among the inferred populations and geography. That is, there is a clear correlation between genetic relationship and distance between populations.

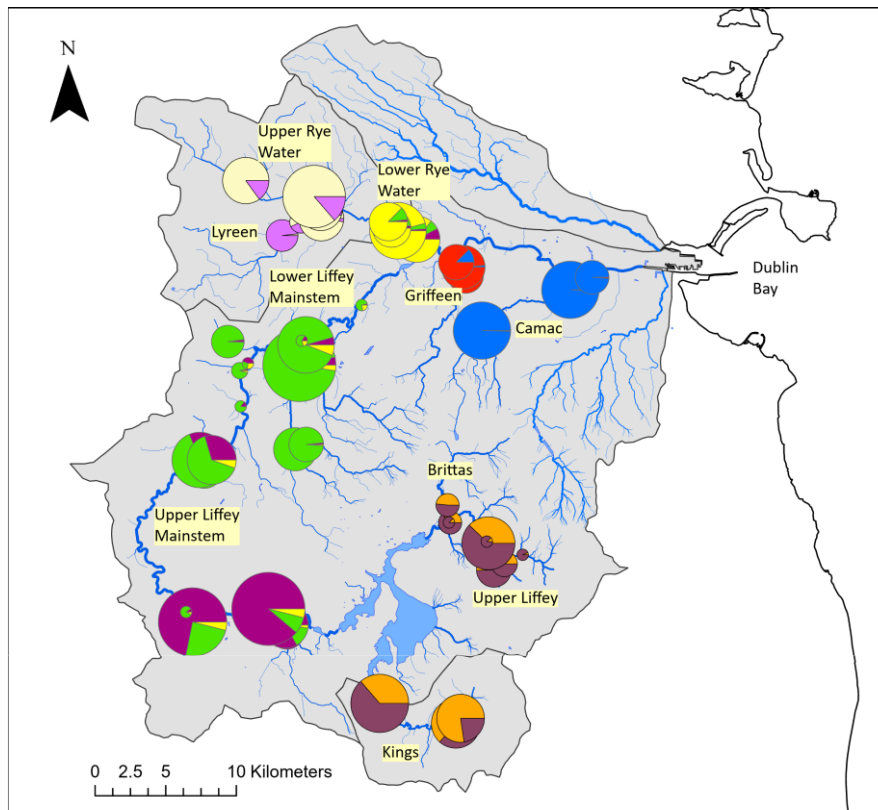


Figure 5. Graphical illustration of the ten Liffey River catchment brown trout populations inferred by STRUCTURE hierarchical analysis.

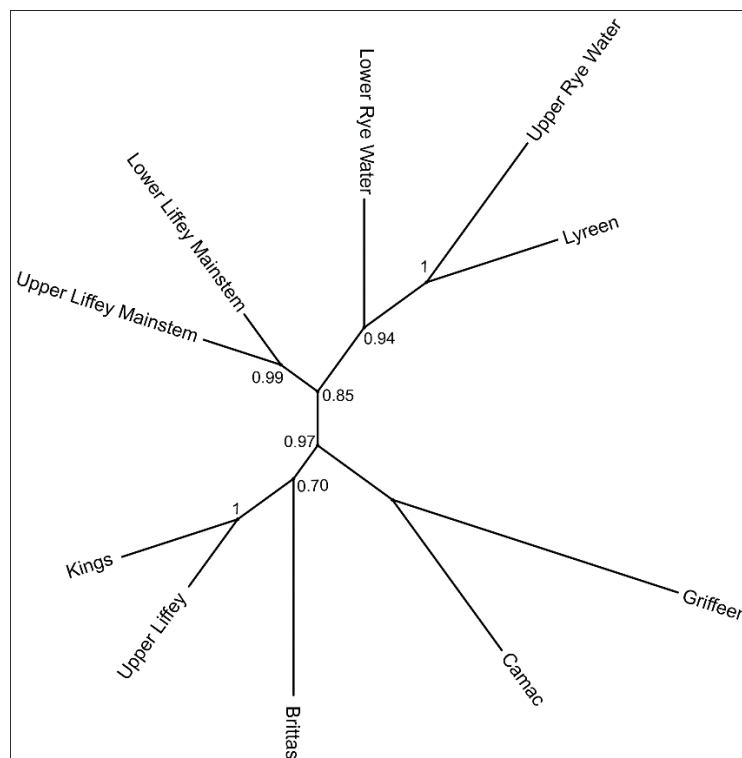


Figure 6. Unrooted NJ phylogenetic tree based on Nei's DA (1983) genetic distance illustrating the relationship among the Liffey River catchment inferred populations. Values at nodes indicate bootstrap support from groups.

Summary diversity basic statistics for the ten populations are displayed in Table 2. Average allele richness values among inferred populations ranged from 5.67 (Upper Rye Water) to 0.69 (Camac River) with an average value of 6.37. The overall levels of both observed and expected heterozygosity are also similar among populations (avg. H_o = 0.62; avg. H_e = 0.65). These genetic diversity values are similar to what has been reported for other similar brown trout population genetic studies in Ireland and elsewhere (e.g. Delanty *et al.* 2020, 2022, Prodöhl *et al.* 2019).

Table 2. Summary statistics of the Liffey River catchment inferred baseline populations identified in this study. N = number of samples that have amplified for over 70% of loci and consequently used for data analyses; Ar = average allelic richness; H_o and H_e = observed and expected heterozygosity; HWE – test for conformance to Hardy-Weinberg Expectations (ns = non-significant).

Inferred Population	N	Ar	H_o	H_e	HWE
Brittas River	48	6.2	0.63	0.67	ns
Upper Liffey	51	6.6	0.64	0.65	ns
Kings River	62	6.57	0.6	0.64	ns
Upper Liffey - Main Stem	174	6.52	0.61	0.63	ns
Lower Liffey - Main Stem	116	6.53	0.63	0.64	ns
Lower Rye Water	68	6.38	0.62	0.65	ns
Upper Rye Water	76	5.67	0.6	0.61	ns
Lyreen	18	6.22	0.59	0.66	ns
Griffeen River	41	6.47	0.66	0.67	ns
Camac River	59	6.63	0.69	0.7	ns

The overall levels of population divergence among inferred populations from the Liffey River catchment system was F_{ST} = 0.052 (95% C.I. 0.048-0.056). This value is well in agreement to what has been observed among brown trout populations in other systems in Ireland and elsewhere (e.g. Delanty *et al.* 2020, 2022, Prodöhl *et al.* 2019). Pair-wise F_{ST} and D_{Jost} estimates and associated 95% CI (measuring statistical significance) between all ten STRUCTURE inferred populations are displayed in Table 3. As expected, a strong correlation (r = 0.97) was observed between both statistical measures of genetic differentiation. Genetic differentiation (D_{Jost}) between populations ranged from 0.019 (Kings River vs Upper Liffey) to 0.209 (Kings River vs Griffeen). The average pairwise genetic differentiation between populations was ca 0.099.

Table 3. Pairwise (A) F_{ST} and (B) D_{Jost} 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 genetic divergence between population in comparison to “blue” that indicates lower levels. With minor exceptions, all pair-wise population comparisons were found to be significant.

A) F_{ST}

	Brittas River	Upper Liffey	Kings River	Upper Liffey Main Stem	Lower Liffey Main Stem	Lower Rye Water	Upper Rye Water	Lyreen River	Grifeen River	Camac River
Brittas River	-	0.031-0.06	0.03-0.057	0.043-0.062	0.043-0.062	0.049-0.071	0.07-0.097	0.042-0.075	0.063-0.095	0.027-0.047
Upper Liffey	0.044	-	0.009-0.027	0.031-0.048	0.032-0.052	0.043-0.067	0.067-0.093	0.035-0.073	0.058-0.096	0.039-0.063
Kings River	0.0426	0.0169	-	0.032-0.05	0.032-0.049	0.052-0.078	0.077-0.106	0.05-0.09	0.078-0.121	0.048-0.075
Upper Liffey - Main Stem	0.0521	0.0385	0.0403	-	0.011-0.021	0.025-0.041	0.052-0.07	0.032-0.065	0.073-0.105	0.044-0.06
Lower Liffey - Main Stem	0.0516	0.0414	0.0395	0.0158	-	0.026-0.042	0.061-0.081	0.03-0.073	0.063-0.095	0.042-0.056
Lower Rye Water	0.0589	0.0536	0.0642	0.0331	0.0336	-	0.035-0.055	0.004-0.047	0.065-0.097	0.044-0.061
Upper Rye Water	0.0824	0.08	0.0914	0.0604	0.0705	0.0442	-	0.012-0.058	0.096-0.127	0.063-0.086
Lyreen River	0.056	0.0516	0.067	0.0462	0.0485	0.021	0.0315	-	0.041-0.089	0.042-0.075
Grifeen River	0.0778	0.0763	0.0978	0.089	0.0797	0.0807	0.1111	0.0626	-	0.04-0.067
Camac River	0.0355	0.0507	0.0609	0.0516	0.0488	0.0521	0.0739	0.0572	0.0525	-

B) D_{Jost}

	Brittas River	Upper Liffey	Kings River	Upper Liffey Main Stem	Lower Liffey Main Stem	Lower Rye Water	Upper Rye Water	Lyreen River	Grifeen River	Camac River
Brittas River	-	0.057-0.116	0.056-0.109	0.069-0.11	0.074-0.113	0.085-0.138	0.12-0.174	0.066-0.143	0.117-0.182	0.035-0.074
Upper Liffey	0.0843	-	0.005-0.038	0.051-0.081	0.053-0.087	0.07-0.115	0.109-0.153	0.041-0.111	0.13-0.2	0.059-0.108
Kings River	0.0805	0.019	-	0.054-0.087	0.046-0.078	0.092-0.143	0.125-0.174	0.079-0.159	0.172-0.25	0.089-0.142
Upper Liffey - Main Stem	0.088	0.0657	0.069	-	0.013-0.03	0.037-0.062	0.081-0.11	0.054-0.108	0.134-0.188	0.065-0.101
Lower Liffey - Main Stem	0.0933	0.0691	0.0604	0.021	-	0.046-0.079	0.096-0.133	0.059-0.133	0.133-0.192	0.068-0.098
Lower Rye Water	0.1095	0.0901	0.1164	0.0488	0.0615	-	0.055-0.091	0.005-0.069	0.134-0.199	0.073-0.111
Upper Rye Water	0.1463	0.1295	0.1494	0.0949	0.1151	0.0711	-	0.015-0.082	0.174-0.229	0.104-0.153
Lyreen River	0.0988	0.0716	0.1146	0.0772	0.0924	0.0262	0.044	-	0.094-0.191	0.071-0.151
Grifeen River	0.1484	0.1648	0.2089	0.1605	0.1629	0.1661	0.1998	0.1379	-	0.072-0.132
Camac River	0.0527	0.0827	0.1147	0.0822	0.0818	0.0906	0.1275	0.1062	0.0989	-

The results of the self-assignment test are summarized in Figure 7. With the exception of the Lyreen River (47.1% self-assignment), all other populations exhibited a high level of assignment – averaging at 82.6%, ranging from 71.7% (Upper Liffey) to 98.6% (Upper Rye Water). This overall high level of self-assignment both demonstrate the power and validate the use of the identified baseline for the assignment of individuals of unknown origin. It is worth noting that miss-assigning individuals invariably assign to genetically (and geographically) related populations. Thus, these miss-assignments, most likely, reflect a degree of straying, a common salmonid behaviour.

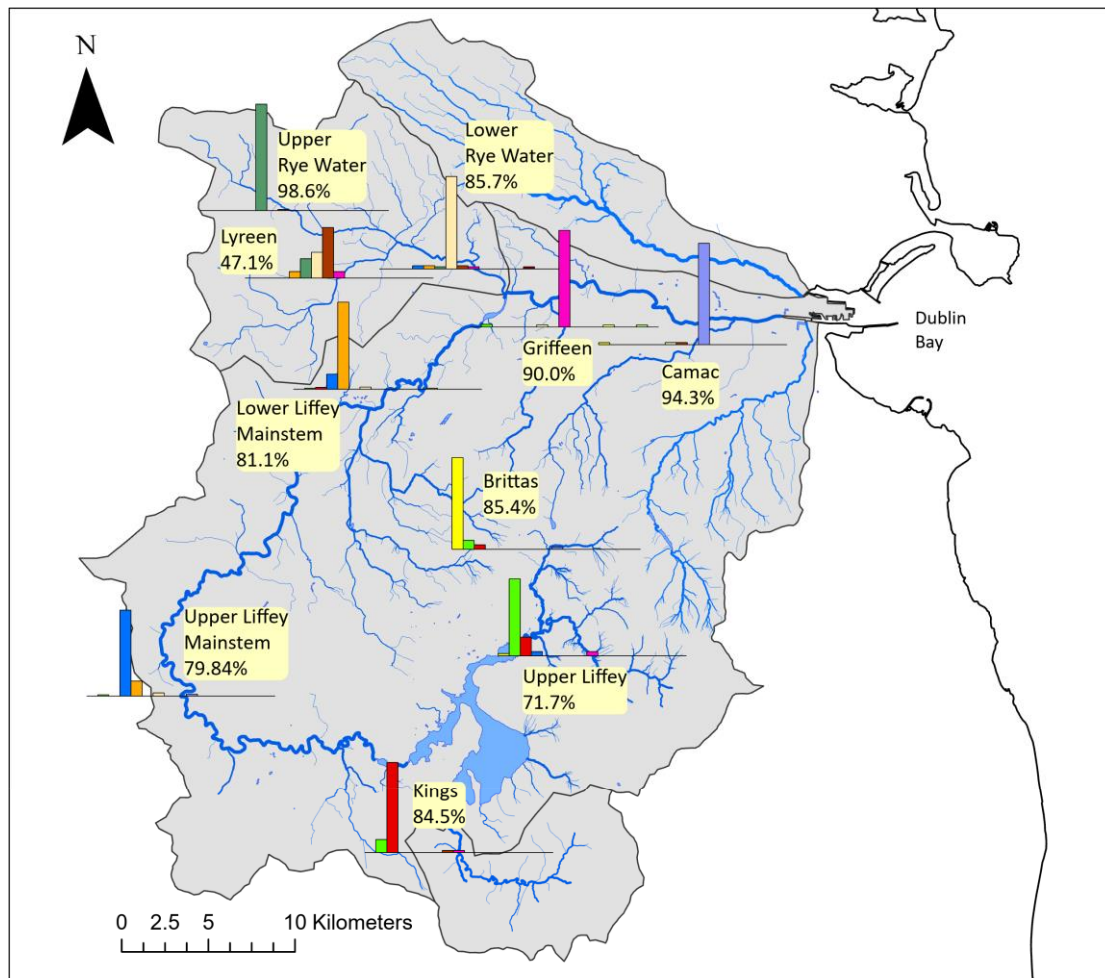


Figure 7. Graphical summary (in %) of correct self-assignment (ONCOR) to the ten brown trout populations identified in Liffey River catchment system.

Results of the assignment ($P > 0.7$) of adult fish to STRUCTURE inferred baselines are summarised in Fig. 8. In general, there was a high level of correlation between site of capture of adult fish and assigned population. Thus, with a few exceptions invariably involving a low number of individuals, adults often assigned to a population close to their location of capture. The overall contribution of STRUCTURE inferred populations to the adult stock for the individual river systems are shown in Fig. 9. The larger contributors, to the adult trout population for the whole Liffey River catchment, are the Upper and Lower Liffey main stem populations with 37% and 21.5% respectively. It is important to note that 13% of the adults ($N = 69$) were not assigned with confidence (i.e. $P < 0.7$) to any of the population baselines identified in this study. Good genetic data was obtained for nine of the ten sea trout identified during this study. Of these, two assigned to the Upper Liffey mainstem, two to the Lower Liffey mainstem, one to the Lower Rye Water, and one to the Griffeen. Of the remaining three, two assigned to the Dodder River and one to the Tolka. (Hynes *et al.* in prep).

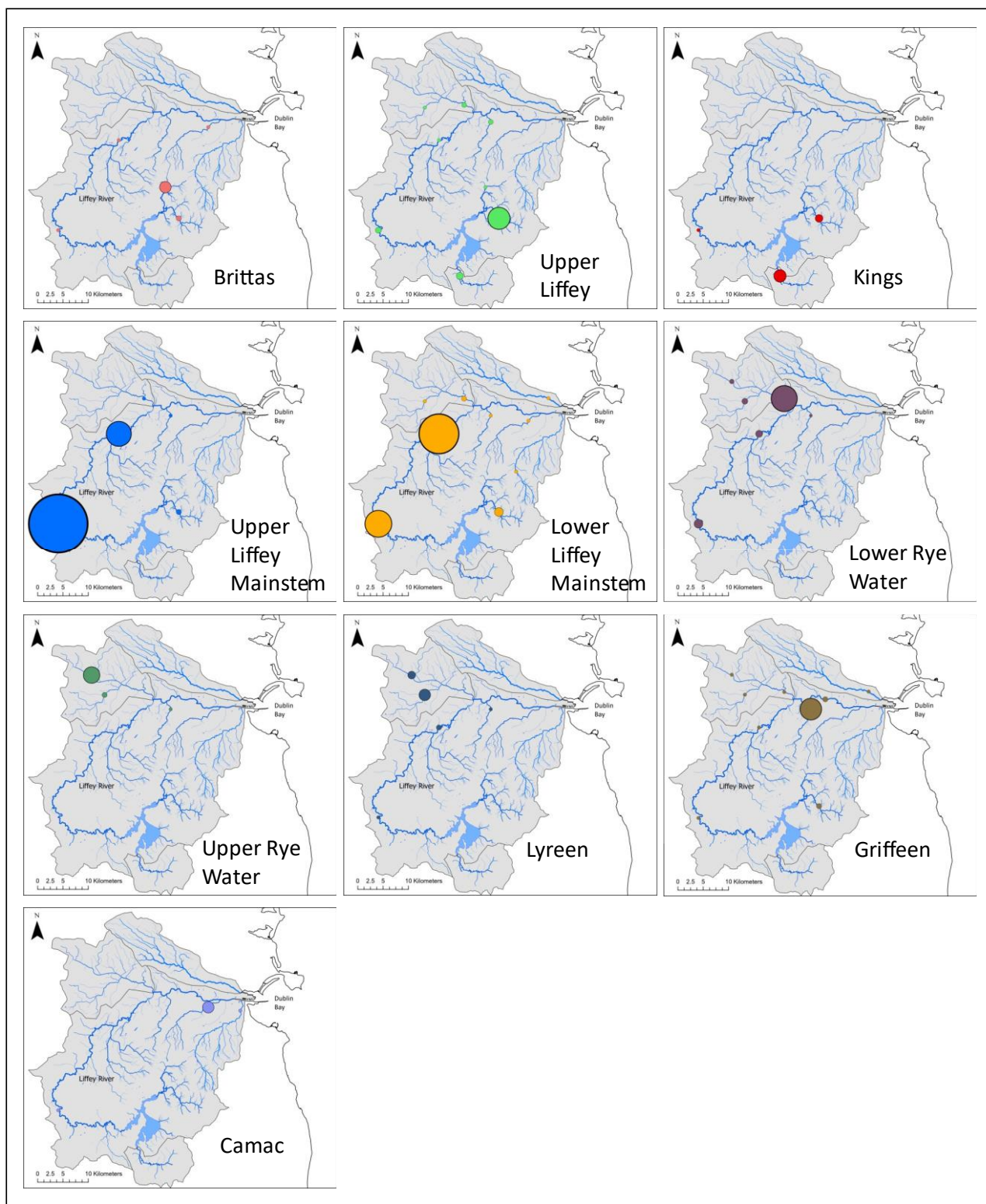


Figure 8. Population baseline contribution to adult stock in relation to site of capture. Each map illustrates assignment to a particular baseline population. The coloured pies indicate site of capture of adult fish in relation to population baseline. In each case, the size of the pies are proportional to the number of adult fish caught.

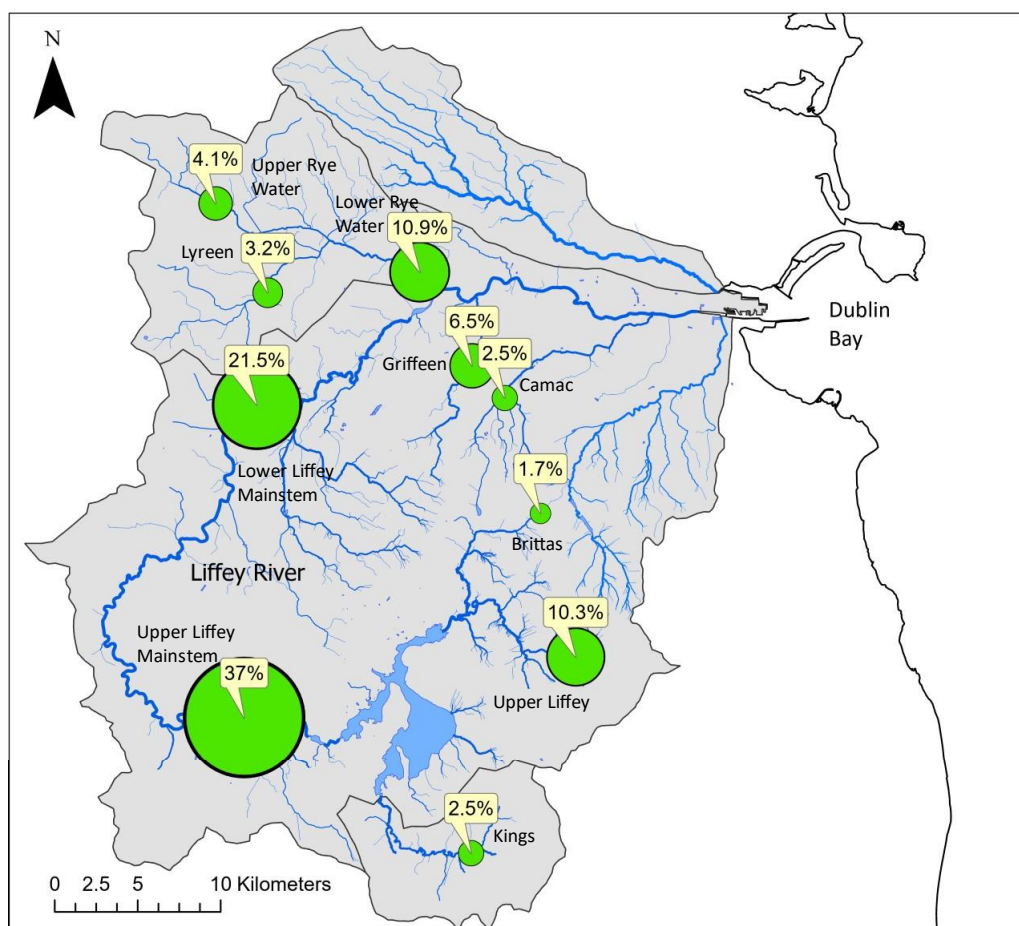


Figure 9. Overall population baseline contribution (in %) to adult stock caught in the Liffey River catchment system.

Liffey Main Channel Adult Brown Trout

The previous section reports on the assignment (population of origin) of all adult trout sampled from the entire catchment. That is, adult fish caught both, in the several tributaries of the Liffey River in addition to those in the main channel. In this section, only adults sampled from the Liffey main channel (i.e. Upper Liffey Mainstem, Lower Liffey Mainstem and Lower Liffey Channel) are examined. In total, 196 adult fish were sampled from the three regions of the Liffey main channel (Table 4). Most of these (N = 118 - 60%) were caught in the Upper Liffey Mainstem region. Considering the whole Main Channel, the majority of the adult fish (N = 126 - 64%) assign to the Upper Liffey Mainstem baseline population and 25% (N = 49) assign to the Lower Liffey Mainstem population (Table 4). Thus, the importance of the Upper Liffey Mainstem (and its tributaries) to the adult stock of the Liffey main channel is significant.

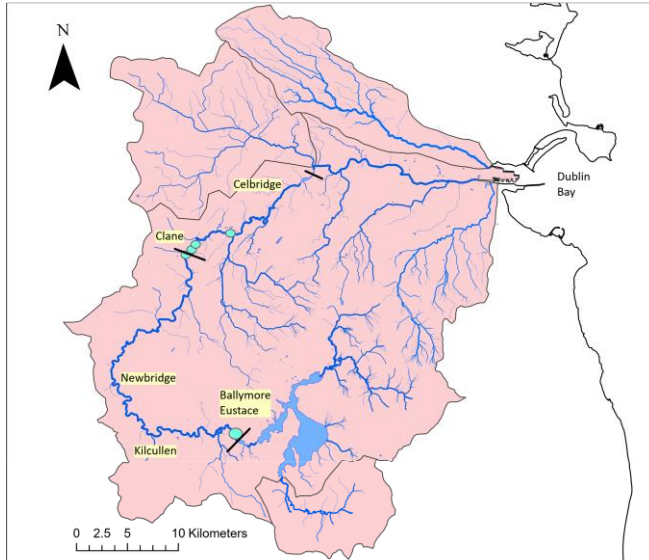
Results also indicate that adult fish tend to remain close to the area of their baseline population (Fig. 10). Thus, 83% of the adults caught in the Upper Liffey Mainstem assigned to the baseline population of that region. A small proportion (14%) of the adult fish caught in the Upper Liffey Mainstem assigned to the geographically close Lower Liffey Mainstem population baseline. This suggests at least some degree of migratory movement, a common trait of brown trout. Only a very small number of adults caught in the Upper Liffey Mainstem assign to other areas (Table 4).

Table 4. Assignment of adult brown trout caught in the Liffey main channel.

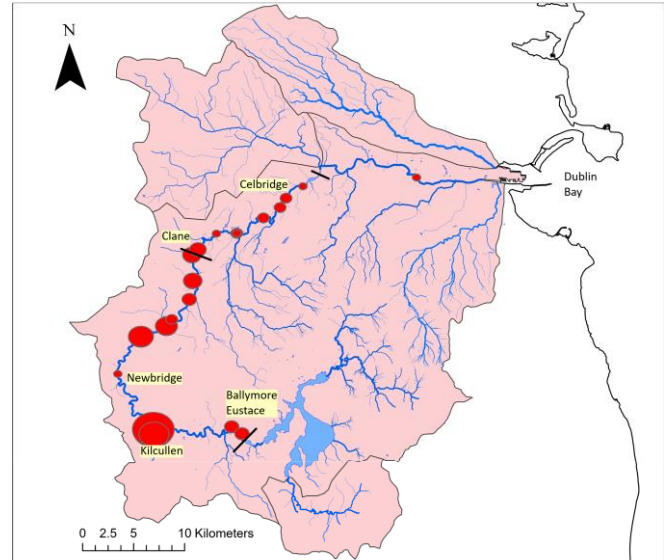
River Liffey Main Channel Adult Assignments	N	Camac	Griffeen	Rye Water	Lower Liffey Channel	Lower Liffey Mainstem	Upper Liffey Main stem	Upper Liffey (u/s Poulaphouca)
Upper Liffey Main Stem ¹	118	-	1 (1%)	1 (1%)	-	16 (14%)	98 (83%)	2 (2%)
Lower Liffey Main Stem ²	59	-	-	2 (3%)	-	29 (49%)	24 (41%)	4 (7%)
Lower Liffey Channel ³	19	-	2 (11%)	9 (47%)	-	4 (21%)	4 (21%)	-
Main Channel ⁴ (d/s Poulaphouca)	196	-	3 (2%)	12 (6%)	-	49 (25%)	126 (64%)	6 (3%)

1 - BME - Millicent Br); 2- d/s Millicent to Celbridge; 3- (d/s Leixlip Dam); 4- BME - to sea

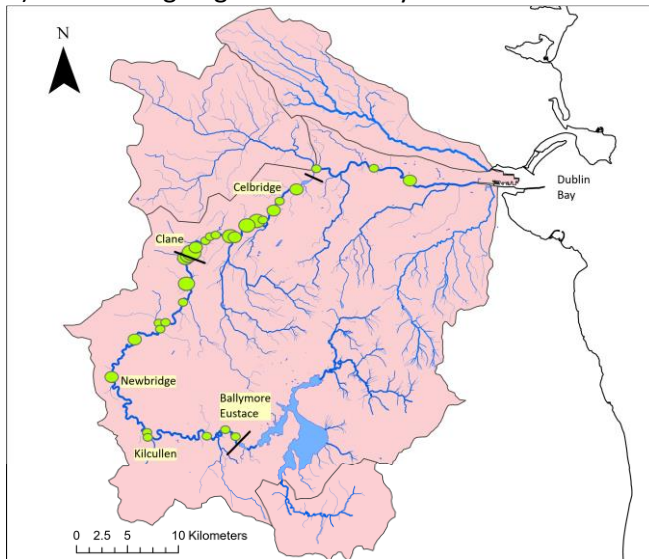
A) ● Fish assigning to Upper Liffey baseline



B) ● Fish assigning to Upper Liffey Mainstem baseline



C) ● Fish assigning to Lower Liffey Mainstem baseline



D) ● Fish assigning to Lower Rye Water baseline

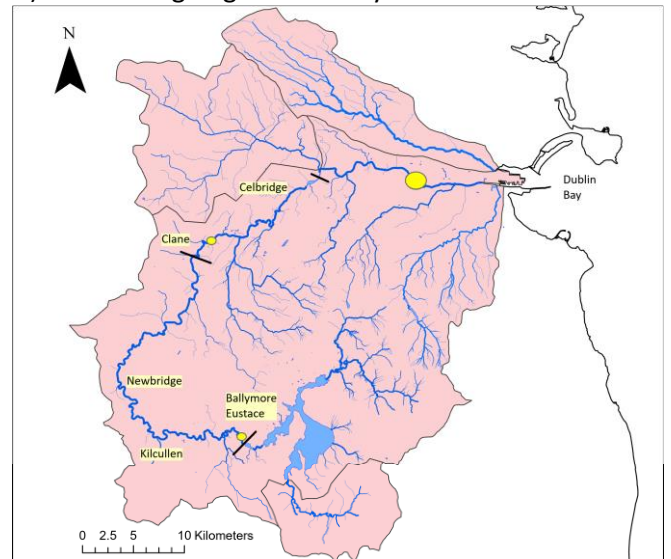


Figure 10. Capture site and origin (baseline population) of adult brown trout caught in the Liffey main channel. In each case, pie sizes are proportional to number of adults caught per site.

Similarly, 49% of the adult fish caught in the region downstream of Millicent Br. and upstream of Leixlip Reservoir (Celbridge) – Lower Liffey Mainstem, assigned to local baseline population (and its tributaries). That is, they assign to the Lower Liffey Mainstem baseline. An additional 41% of the adult fish caught in this region again assigned to the geographically close Upper Liffey Mainstem baseline population. These results confirm and further highlight the importance of the Upper Liffey Mainstem (and its tributaries) to the brown trout adult stock of the region ranging from BME to Celbridge.

Results from the assignment of adult trout taken downstream of Leixlip suggest that the Rye Water is a substantial contributor with 47% adult assignment. It is important to note, however, that the number of samples available from this region was small (19) and eight individuals were physically identified as sea trout. Other baseline populations include the Upper and Lower Liffey Mainstems and the Griffeen.

5. Summary Discussion

Over the past two centuries the River Liffey and its tributary rivers have been subjected to several environmental disturbances that have contributed to the alteration of the natural aquatic 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, large scale hydroelectric power schemes, farming activities and agricultural run-off, introduction of alien species, fish stocking and arterial drainage of river networks. 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. Over the past few decades, there have been efforts to mitigate environmental issues (e.g., habitat improvement work, modifications of man-made barriers and weirs and water quality improvement). The results presented in this study suggests that brown trout within the Liffey River catchment system were able to cope and adapt to changing environments. Thus, indicating a noteworthy level of natural resilience from the wild Liffey brown trout, confirming what has been found in other similar investigations focusing on systems impacted by human mediated activities (L. Sheelin, Delanty *et al.*, 2020 and L. Corrib, Delanty *et al.*, 2022).

This study focused on five main objectives, each of which is reviewed here.

1) To describe the contemporary brown trout population genetic structure of the Liffey River catchment system

A substantial level of population sub-structuring was observed within the Liffey River catchment system, with a total of ten genetically distinct brown trout populations being identified from the samples analysed. Most likely, this pattern of population structuring reflects a combination of historical (i.e., natural colonisation patterns) and contemporary factors (adaptation to human mediated environmental pressures). The levels of genetic divergence observed suggest limited gene flow among contemporary populations, and a clear pattern of isolation by distance (i.e., higher levels of gene flow between geographically close populations).

Three of the identified populations are above Poulaphouca dam and reservoir, and two above the man-made waterfall and artificial lakes at Carton Estate (Fig. 1). Considering that the waterfall at Golden Falls was known to be impassable to salmonids prior to construction of the dam, it is not surprising that brown trout populations above the falls (and now Poulaphouca dam and reservoir) are genetically distinct as they have been isolated for longer periods of time. These genetic differences are, thus, mostly the outcome of natural processes (population isolation resulting in stronger genetic drift isolation and adaptation to local environmental conditions).

While natural processes cannot be dismissed to explain the genetic differences identified in the two populations above the man-made waterfall and artificial lakes at Carton Estate, it is likely that differences have been further exacerbated by these artificial barriers to migration (population isolation resulting in stronger genetic drift). The Liffey main channel itself currently harbours two genetically distinct populations. It is difficult to know whether these populations have always been naturally present in the system, or whether they are the result of recent evolutionary history driven by human mediated habitat alteration. It is well known that the many barriers and weirs (in addition to the Leixlip hydroelectric power station), constructed along the main channel have greatly changed the brown trout natural habitat by restricting the potential for migration. While sea trout are still recorded in the system, numbers are now very low. Only ten individuals were morphologically identified as sea trout during this study.

Salmon, historically, would have had greater access to the Liffey mainstem (up to Poulaphouca Falls) and were present in greater numbers prior to the building of the ESB generating station, dam and reservoir at Leixlip (Went, 1945-1948). Thus, brown trout would have shared the Liffey mainstem resources (for spawning and nursery waters) like many other Irish large river systems. Since the 1940's salmon access to spawning and nursery grounds above Leixlip Reservoir have been restricted and passage through the dam has been through artificial means of a hydraulic lift operating at the ESB generating station (ESB, 2022).

The Liffey catchment differs from many other large river systems in that many of its tributary catchments and wetted areas are relatively small. The Morell and Rye Water sub-catchments being the only significant tributaries to the main channel. There are, however, significant amounts of salmonid spawning opportunities present in areas of shallow glide and riffle sections of the main channel (especially between BME and Straffan), as noted in the 2021 'Fish Stock Survey of the River Liffey' report (Delanty *et al.*, 2022).

While propensity for anadromy (i.e. genetic drive) still exists within the brown trout populations of the Liffey River catchment system, given the many obstacles to sea migration, it is likely that there is now strong selection against this life history strategy. Thus, contemporary brown trout populations are now characterised by fish undertaking limited migration from spawning to feeding grounds. The modification of barriers and weirs over the past few decades have facilitated movement (and at least a certain level of gene flow), however the genetic integrity of contemporary populations is now likely maintained by the strong homing behaviour of brown trout, a well known life history trait of salmonids in general.

While brown trout from some of the Liffey tributaries form genetically distinct populations (Griffeen, Camac and Rye Water), no genetic differences were observed among brown trout from others (Morell, Gollymochy, Awillyinish, Mill Stream and Ardinode/Lemonstown). Brown trout from these tributaries are most likely part of large *metapopulations* centred in the main channel regions into which they join (Upper Liffey mainstem or Lower Liffey mainstem). Therefore, it is not possible to determine the relative contribution these tributaries make to the over-all adult brown trout fishery of the main channel. Metapopulations comprise several local populations that are largely independent, but interconnected by usually large levels of gene flow (i.e. large levels of straying fish) and, linked to extinction and recolonisation events (Hanski, 1998). Thus, it is often the case that local stock declines brought about by several factors (e.g., fish kills, water quality issues) are naturally mitigated by fish moving from other areas of the metapopulation (i.e. effectively individuals sharing similar genetic makeup). Metapopulations are characterised by weak genetic differentiation and absence of isolation by distance (Garant *et al.* 2000), all features noted in brown trout inhabiting these tributary rivers of the Liffey main channel. For the purposes of conservation and management, the *metapopulation* (and not individual components, i.e., brown trout from associated tributaries) should be the unit of interest (i.e. targets of management and conservation plans).

2) To describe the relative contribution of identified populations to the adult brown trout fishery (Genetic Stock Identification).

Both the Upper Liffey Mainstem and Lower Liffey Mainstem baseline populations (metapopulations) were found to be the main contributors (~89%) to the adult brown trout stock of the main stem fishery. With a few exceptions, most adult fish seem to concentrate in areas close to their respective river baselines. The Camac and Griffeen rivers were not found to contribute significantly to the main Channel adult brown trout stock (2%), especially in the Lower Liffey Channel. It is important to note, however, that this result may be biased given the low number of adults available to the study from the Lower Liffey Channel or a sea trout element. Nevertheless, both rivers seem to support limited resident brown populations.

While the Rye Water is the largest sub-catchment of the Liffey River catchment system, its contribution to the main channel adult brown trout stock seems to be limited. Overall only 6% (12 adult fish) of the main channel population were found to assign to the Rye Water. The majority of adult fish from the Lower Main Channel (d/s of Leixlip) were Rye Water fish (47% or 9 fish) (Table 4).

The overall proportion (13%) of unassigned adult fish (i.e., $P < 0.7$), from across the whole Liffey catchment, needs to be interpreted 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). The proportion of adult fish which could not be assigned to a baseline population (i.e., with a p value of < 0.7), noted from the Liffey main channel only (from BME to Leixlip), is 14.5%. The high levels of movement of adult fish noted along the Liffey main channel, especially between the Upper Liffey Main Stem and the Lower Liffey Main Stem, was not unexpected. This has been also observed in other large river main channels such as the Boyne River and Suir River (Massa-Gallucci & Steffano, 2011).

3) To determine the potential impact(s) of the stocking history on the wild brown trout populations

Notwithstanding the well documented extensive stocking history within the Liffey River catchment system, there was no evidence to suggest that there has been any long-term influence (i.e. genetic introgression) of hatchery trout on the current wild brown trout population. Thus, no hatchery derived fish was identified among the adults sampled for this study. This observation is surprising given the still ongoing stocking in parts of the system. In summary, there is no evidence indicating any genetic impact(s) resulting from the known extensive stocking history from Roscrea farm fish into the Liffey catchment brown trout wild stock. These results are in agreement with what has been observed in other brown trout genetic based surveys in Ireland (Delanty *et al.*, 2020 and Delanty *et al.*, 2021). As clearly shown here (Fig 3), 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 and Delanty *et al.* (2021) for L. Corrib. Farm-reared brown trout are different from wild brown trout in several ways. They are genetically different because of founding effects, and subsequent domestication in the farm environment, involving artificial selection, relaxed natural selection, and genetic drift. They also differ in their learning opportunities especially in relation to feeding and anti-predator behaviour. Given all these differences, it is not surprising that stocking with farm-reared trout results in little or no long-lasting impact on wild populations either in terms of increasing fish numbers or in producing

genetic changes (Ferguson, 2007). Given these differences, when stocked as fry, juveniles or adults, 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.

4) To determine the impact of barriers (natural and manmade) to fish migration and influence on present day stock structure

As summarised in Objective 1, most populations in the system can be explained by the presence of contemporary and/or historical barriers. Excluding major obstacles to natural migration (e.g., dams, etc), there is no major evidence to suggest that man-made barriers are currently preventing fish movement in the Liffey.

Barriers are an issue worthy of note (Donovan *et al.*, in prep.) – 3 ESB hydro dams and several main channel weirs (in particular those at Athgarvan, Morris Lattin and Wrens nest) and Carton Estate on the Rye Water.

The River Rye Water was dammed at Carton house (Fig. 1) in the early 1800's through the creation of two artificial lakes with a waterfall separating the two lakes and a weir at the outflowing point of the lower lake (<https://www.intel.ie/content/dam/www/public/emea/ie/en/documents/river-rye-web.pdf>). This man-made barrier was impassable to salmonids and prevented any further upstream migration of salmon and trout to the remaining Rye Water system. A fish pass was built into the waterfall around 2008, and the river upstream stocked out with hatchery reared salmon parr (~100,000) over a period of approximately three years (<https://www.irishtimes.com/news/anglers-pay-tribute-1.11368>). However, no salmon have been recorded above the modified weir from during the course of several electrofishing surveys carried out by IFI (Holmes *et al.*, 2008-2018; IFI, 2018). As summarised under Objective 1 these man-made barriers (lakes and weir) which have been present for almost 200 years, is likely to explain genetic differences observed between brown trout from the lower Rye Water and from both upper Rye Water and Lyreen.

The fish pass at Leixlip dam is likely to be the reason why some fish from Rye Water, Camac, Griffeen can be found above the dam. It is possible that adult trout along with salmon are caught in the fish lift system during natural migration and end up in the Liffey mainstem. Indeed, 10 adult fish that were caught as adults above dam were assigned to baseline populations below the dam, including Rye Water, Griffeen, Camac and Upper & Lower Liffey Mainstems.

5) To identify potential populations producing anadromous brown trout (i.e. sea trout) within the Liffey catchment system

As summarised under Objective 1, only 10 sea trout were identified in this study. Good quality genetic data was generated for nine individuals. Of these, two assigned to the Upper Liffey mainstem, two to the Lower Liffey mainstem, one to the Lower Rye Water, and one to Griffeen. Of the remaining three, two assigned to the Dodder River and one to the Tolka. The latter observation indicates at least some level of straying of sea trout among these three main river catchments (Hynes *et al.* in prep). As discussed above, while brown trout from the Liffey River catchment still can produce sea trout, given the changes to their natural environment, it is likely there is now selection against sea migration with increasing survival (biological fitness) for individuals that remain in freshwater. Although the adult sea trout sample size, from the Liffey, was quite small three of these fish did assign to external populations, i.e., the Dodder and Tolka systems.

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