Genetic Evaluation of wild Atlantic Salmon Populations in Lochaber: Project Report, February 2026

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Introduction

The Lochaber region in northwest Scotland contains thirteen major river systems which contain wild Atlantic salmon (*Salmo salar*) populations. Several of these support important recreational fisheries. The wild Atlantic salmon populations in Lochaber have declined in recent years, echoing a similar trend across Scotland and throughout northern Europe. Reasons for these Atlantic salmon population declines are not fully understood, but they are thought to result from a combination of factors that include changing conditions in the marine environment. Atlantic salmon aquaculture – active on the west coast of Scotland and generally using farm strains with Norwegian ancestry – may be one of the contributary factors. Amongst other impacts it presents a potential genetic risk to native wild Atlantic salmon populations due to escaped aquaculture fish hybridizing with wild fish.

Proper management of wild Atlantic salmon populations requires estimations of the number of breeding adults, and identification of escaped aquaculture fish or their offspring within the populations. Genetic tools can provide the means to do both. Previously, we used a panel of highly variable genetic markers to estimate the minimum number of breeders that produced fry and parr sampled from several Lochaber rivers (Loy, Lundy, Moidart, Upper & Lower Shiel, Scaddle, Nevis & Roy) in 2021, 2023 and 2024. We used the same markers to assess levels of aquaculture ancestry and estimate sibship effective number of breeders (Nb_{sib}), a standardized measure of the genetic size of the annual breeding population. This report builds on this study by applying the same analyses to fry collected from similar parts of the Loy, Lower Shiel, Nevis and Roy in 2025.

Sampling

Tissue samples were collected in August 2025 by the Lochaber Fisheries Trust. Putative 0+ fry (length 39-76 mm) were caught by electrofishing at multiple sites in the Lochy, Shiel, Nevis and Roy Rivers, most of which overlapped the previous sampling sites (Table 1, Figure 1). These rivers all support wild-breeding Atlantic salmon; at some point in their recent past they are also likely to have been stocked however IBFC was not provided with any stocking information. Small caudal fin clips were taken from the sampled fish and preserved in pre-labelled tubes containing 97% ethanol. All electrofishing and tissue collection was performed following standard protocols under required licences and permissions.

DNA extraction and genotyping-by-sequencing

The fin clip samples were received by the Institute for Biodiversity and Freshwater Conservation in September 2025. All samples were processed in 96-well plates with three 'blank' control wells (containing no salmon tissue) on each plate. DNA was extracted from approximately $2mm^2$ of each fin clip using HotSHOT alkaline lysis (Truett *et al*. 2000). Each sample was genotyped for a panel of 88 short tandem repeat genetic markers ('microsatellites'). Markers were amplified in two separate multiplex PCR reactions containing the following: 3µl 2x Qiagen Type-IT multiplex master mix, 0.3µl primer multiplex mix (45 or 46 primer pairs at a mean concentration of 1µM per primer), 2.7µl diluted DNA. Thermocycling conditions were: 95°C for 15min, 25x [94°C 30s, 57°C 3min, 72°C 30s], 72°C for 10min. The two sets of PCR products were pooled for each sample and diluted 40x with water. Eight 96-well plates were combined for each DNA sequencing run. Sample-specific forward and reverse index combinations and Illumina sequencing tags were added to each sample (including blanks) in 5µl PCR reactions using the following protocol: PCR mix - 2.35µl H_2O, 0.5µl 10x buffer, 0.25U Taq DNA polymerase, 0.1µl dNTPs (10µM each), 1µl forward and reverse index mix (1µM per index); 1µl diluted multiplex PCR product; thermocycler conditions - 98°C for 2 min, 20x [98°C 10s, 62°C 30s, 72°C 15s], 72°C for 10 min. Product for all samples was pooled into a single library, and purification and fragment size selection was performed using Agencourt AmPure XP beads. The concentration of the pooled library was measured via fluorometry using a Qubit with a high-sensitivity kit and standardized. Each pooled library was single-end sequenced on an Illumina MiSeq using Illumina V3 sequencing chemistry (150 cycles), with sequence reads demultiplexed to individual samples on the basis of their sample-specific indices and output in fastq format.

Statistical analysis

Microsatellite genotypes were called from DNA sequence reads using MEGASAT (Zhan *et al.* 2017), using an IBFC standard pipeline. Any brown trout or first-generation trout-salmon hybrids in the dataset were identified from a known combination of non-amplification of certain microsatellite loci with brown-trout specific alleles at other loci. The package rubias (Moran & Anderson 2018) was used in R 4.0.3 (R Core Team 2020) to check for the presence of genetically identical samples (i.e those taken from the same individual fish). Finally, any remaining fish with > 25% missing data and any genetic marker with >25% missing data was removed from the analysis.

The software COLONY 2.0.6.6 (Jones & Wang 2010) was used to infer family structure among the genotyped juveniles and so infer the number of breeders that produced them. COLONY uses a maximum likelihood approach to infer sibling relationships from shared genetic variation, accounting for possible genotyping error. All juveniles collected in 2025 were analysed together. The following parameters were applied: probability of allele drop out 0.001 and other errors 0.001 for all loci; allele frequency not updated; diecious parents; polygamy for both sexes; full sibship scaled; no sibship prior; unknown population allele frequency; combined pairwise likelihood and full likelihood (FLPS) algorithm with medium run length and medium precision. To confirm model convergence, three independent replicate runs were performed with different random seeds. Where results varied, the solution with the largest number of parents was selected. COLONY also generates an estimate of effective number of breeders (Nb_{sib}), based on the inferred sibship relationships. To generate per-location estimates of Nb_{sib} we repeated the COLONY analysis for each separately.

The presence of genetic material from Norwegian-ancestry aquaculture fish (the predominant type used throughout Scotland – although note that one local producer uses a Scottish-ancestry strain) was assessed using STRUCTURE (Pritchard *et al.* 2000). This program infers the ancestral contribution of different genetically distinct groups to a focal individual. We combined the Lochaber genetic dataset with a reference genetic dataset of aquaculture salmon (n = 600). The reference dataset included individuals from three domestic strains commonly stocked on Scotland's west coast, genotyped at IBFC using the same markers, laboratory and bioinformatic pipeline. We ran STRUCTURE specifying two ancestral groups, corresponding to aquaculture fish vs. wild Scottish fish, and applied the following parameters: admixture model with correlated allele frequencies, no prior population information, 100,000 burn-in followed by 150,000 MCMC reps; all other parameters default. As slightly different sets of post-QC markers were retained for this study and the previous study (80 markers shared between both studies), we also included 2021-2024 Lochaber samples in this new analysis to confirm that results from the two studies were comparable. As before, we emphasize that accurate quantification of ancestral proportions is statistically difficult task, particularly when using small numbers of non-diagnostic genetic markers such as microsatellites (Pritchard *et al.* 2007) and when the distinct groups hybridized >2 generations in the past (Pritchard *et al.* 2016). While results of this study can indicate of levels of aquaculture ancestry at different sites, therefore, they should not be considered diagnostic of recent aquaculture ancestry for any individual fish.

Results

Of the 181 samples that were put through the genotyping process, none were brown trout or trout hybrids. Two failed genotyping quality control due to >25% missing data. Five of the 88 microsatellites were also removed due to missing data, leaving 179 individuals (47 from Lower Shiel, 48 from Nevis, 28 from Roy, 56 from Loy) and 82 markers for analysis. (Table 1)

Sibship reconstruction by COLONY identified separate groups of breeding adults for the four different sampling areas, except for two parents that were inferred to have spawned in both Nevis and Roy. Although such long-distance movement of spawning adults is possible, further examination of the results showed low confidence that the apparent Nevis and Roy offspring of these parents were true half siblings. Each of these two candidate parents were therefore split into separate parents for Nevis and Roy. With this adjustment, the following number of parents was inferred for each group of genotyped juveniles: Lower Shiel: 42; Nevis: 41; Roy: 25; Loy: 26. Five full-sib families collected in the Loy (10, 4, 3, 2 and 2 individuals) originated from a single parent that had spawned with five other fish. Only three other full-sib groups (3 or 2 fish) were found in the dataset. Inferred pedigrees for sampled juveniles are shown in Figure 2. For comparison, results for the previous juvenile collections from the same sites are shown in Appendix 1.

Point estimates for Nb_{sib} –– were: Lower Shiel 62 (95% confidence intervals: 43-96); Loy 12 (6-26); Nevis 62 (43-96); Roy 17 (10-36).

Estimation of wild vs aquaculture ancestry using STRUCTURE inferred rather little aquaculture influence among the four sampled regions. Results are shown in Figure 2. Apart from some minor variation in exact ancestry estimates, using a slightly different marker set did not change the results for juveniles collected in 2021-2024 (Appendix 1).

Discussion

Genetic reconstruction of sibships among the 179 genotyped juveniles inferred a total of 384 distinct parents, ranging from 62 in the Lower Shiel and Nevis to 12 in the Loy. As in our previous study despite high aquaculture activity on the west coast of Scotland, our analysis did not infer large amounts of Norwegian aquaculture ancestry in most sampled juveniles.

Previous work by IBFC, applying the same set of markers and COLONY parameters to reconstruct parents for juveniles whose parentage was known, has shown that this approach may slightly underestimate true numbers of parents. Additionally, the number of parents identified is expected to increase with juvenile sample size, although this will approach an asymptote as the true number is reached. In practice, it is impossible to discover the true adult population size from reconstruction of juvenile sibships, even if all juveniles are sampled, because some breeders will produce no surviving offspring (Waples & Feutry 2021). Thus, our parentage reconstruction gives an indication of the **minimum** number of breeding adults only. This includes both sexually mature adults returning from the sea and precocious parr, which can be 30% or more of wild spawners (Saura *et al.* 2008). Comparing our results for 2025 for those estimated from juveniles collected for the same sites in 2021 or 2024 (Appendix 1), we observe rather similar juvenile to inferred parent ratios, despite these previous collections mostly targeting parr. The exception to this was the Loy, where a large number of sampled fry, distributed across several adjacent sampling sites, were the offspring of just a single parent. Although genetic parentage reconstruction can only discriminate the two sexes that contributed to each breeding cluster and cannot identify which is male and which is female, examination of the pedigree figures (Figure 1) reveals several individuals that spawned with multiple members of the opposite sex to produce a relatively large number of offspring. It seems likely that these individuals are multi-seawinter females, which are known to have a large influence on salmon productivity in a river.

References

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Table 1: Details of samples collected and genotyped

River	Trib	Site	Lat	Long	Size range (mm)	N	QC Fail	QC Pass	Previous analysis year
Lochy	Loy	Loy01	783119	212483	49-68	10	0	10	2021 (Mostly Parr)
Lochy	Loy	Loy02	783167	212194	54-65	18	0	18	2021 (Mostly Parr)
Lochy	Loy	Loy03	783404	211490	59-70	12	0	12	2021 (Mostly Parr)
Lochy	Loy	Loy04	783648	211369	55-70	10	0	10	2021 (Mostly Parr)
Lochy	Loy	Loy05	784402	210624	58-65	6	0	6	2021 (Mostly Parr)
Nevis	Nevis	Nev01	773083	212367	57-64	17	0	17	2024 (Mostly Parr)
Nevis	Nevis	Nev02	771285	213065	55-70	21	0	21	2024 (Mostly Parr)
Nevis	Nevis	Nev03	770258	213541	45-56	9	0	9	2024 (Mostly Parr)
Nevis	Nevis	Nev04	769268	214107	59-59	1	0	1	2024 (Mostly Parr)
Roy	Roy	LOC35	789384	231330	63-76	7	0	7	2024 (Mostly Parr)
Roy	Roy	LOC36	791022	233280	62-71	2	0	2	2024 (Mostly Parr)
Roy	Roy	LOC37b	792430	235387	53-68	15	0	15	2024 (Mostly Parr)
Roy	Roy	LOC39	793261	238941	63-68	4	0	4	2024 (Mostly Parr)
Shiel	Lower Shiel	Shi01	768972	167569	56-70	9	1	8	2021 (Mostly Fry)
Shiel	Lower Shiel	Shi02	769221	166438	59-59	1	0	1	2021 (Mostly Fry)
Shiel	Lower Shiel	Shi03	769690	166596	39-63	24	1	23	2021 (Mostly Fry)
Shiel	Lower Shiel	Shi04	769794	166659	43-62	15	0	15	2021 (Mostly Fry)

Figure 1. Juvenile sampling locations 2025

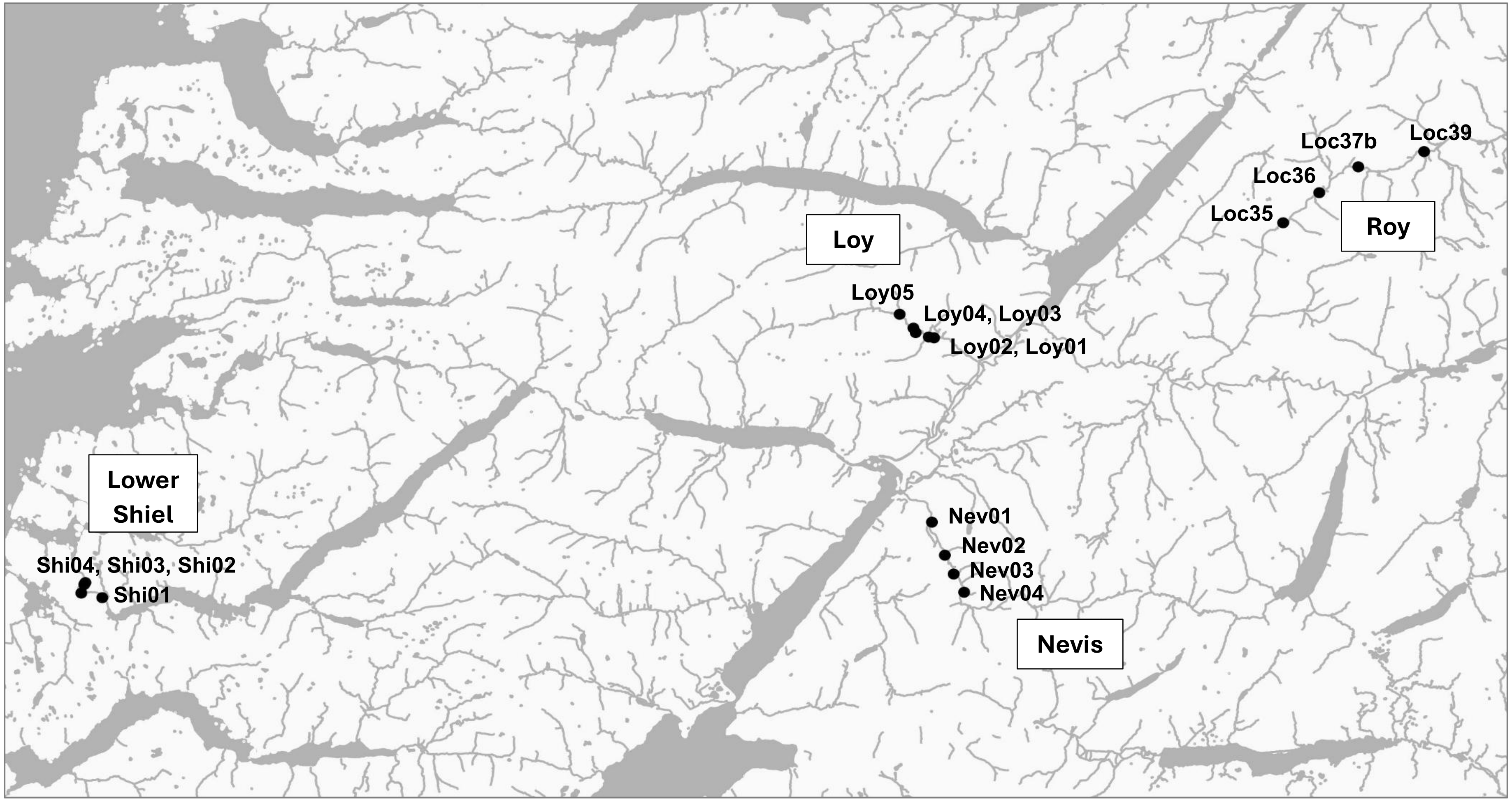
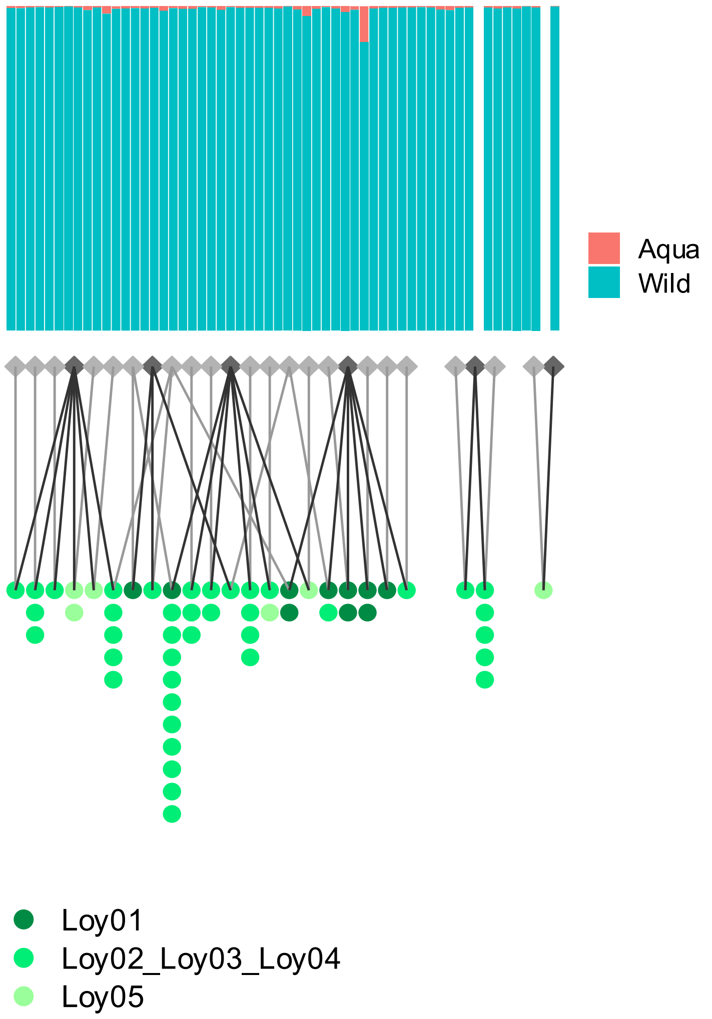
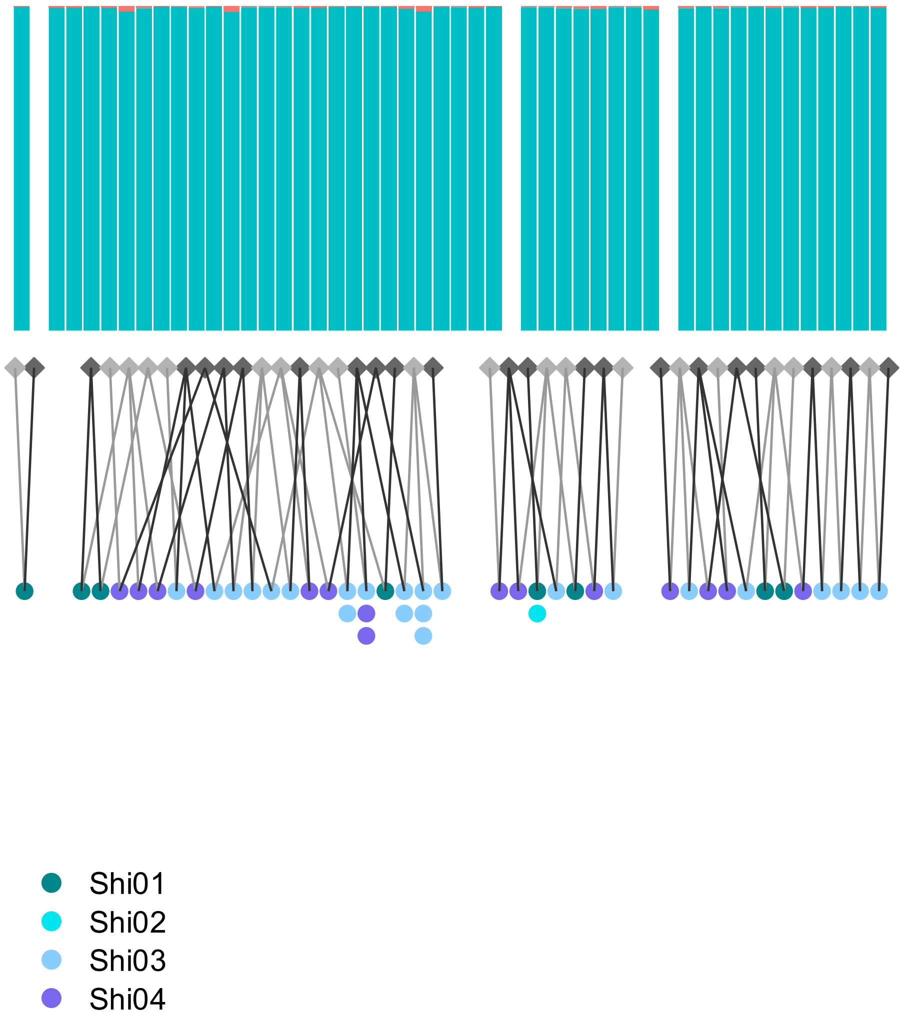


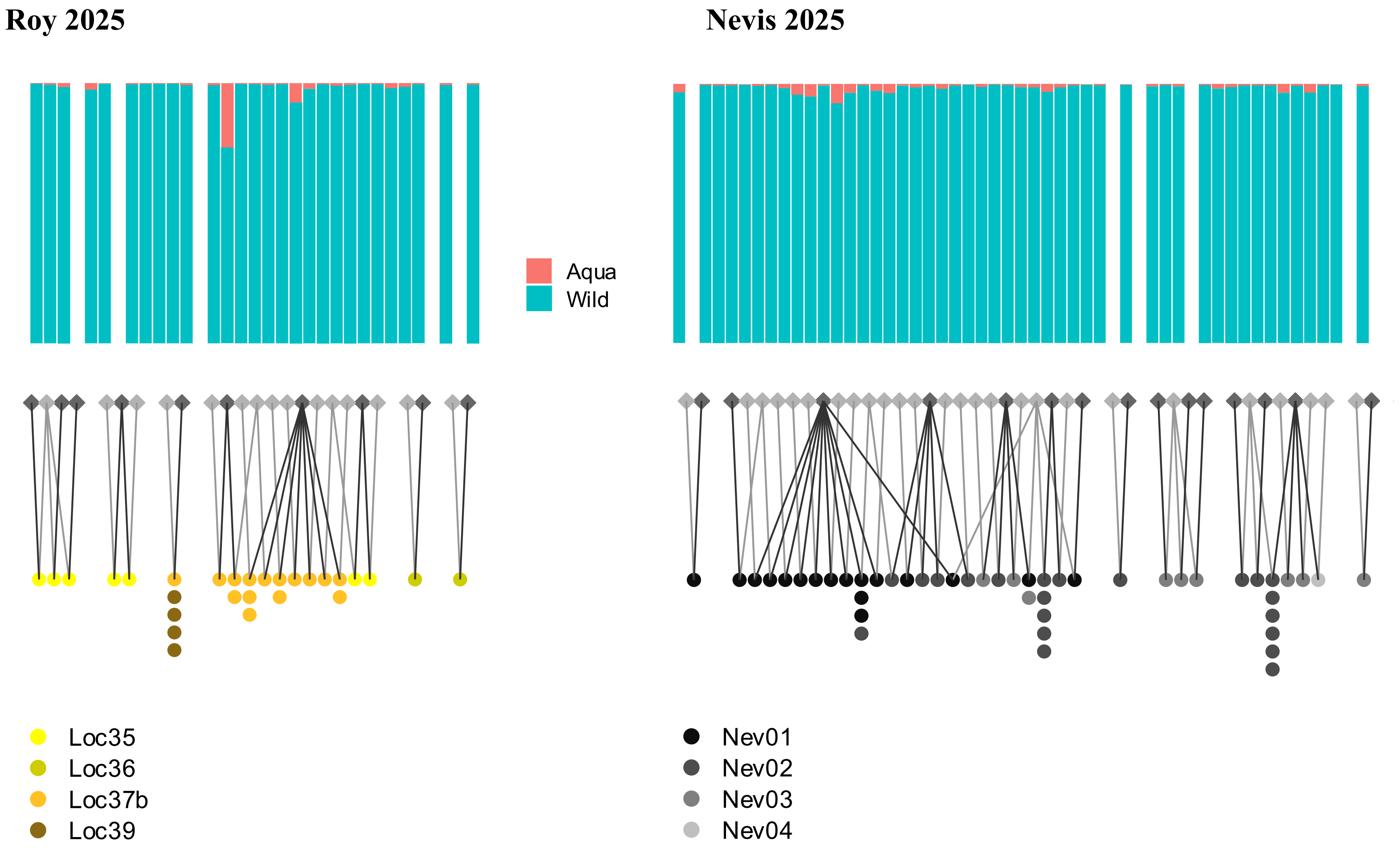
Figure 2 (overleaf). Inferred ancestry of each genotyped fish (top) and inferred pedigree (bottom) with figures in the same order. For ancestry figures, each column represents an individual fish and the colour represents the proportion of their ancestry assigned to wild Scottish or Norwegian-origin aquaculture fish. Note that, using this analysis method, a small amount of aquaculture ancestry (< 1%) is inferred for all wild fish due to statistical noise. For pedigree figures, inferred parents (diamonds, top) are linked by a line to their genotyped offspring (circles, bottom). The two parental sexes are indicated by different shades of grey, however which shade corresponds to male vs. female is unknown and it will vary between breeding clusters. Circle colour indicates offspring sampling site. Offspring arranged in a vertical column are full siblings. Sampled fish are arranged in the same order in the ancestry and pedigree figures.

Lochy (Loy) 2025



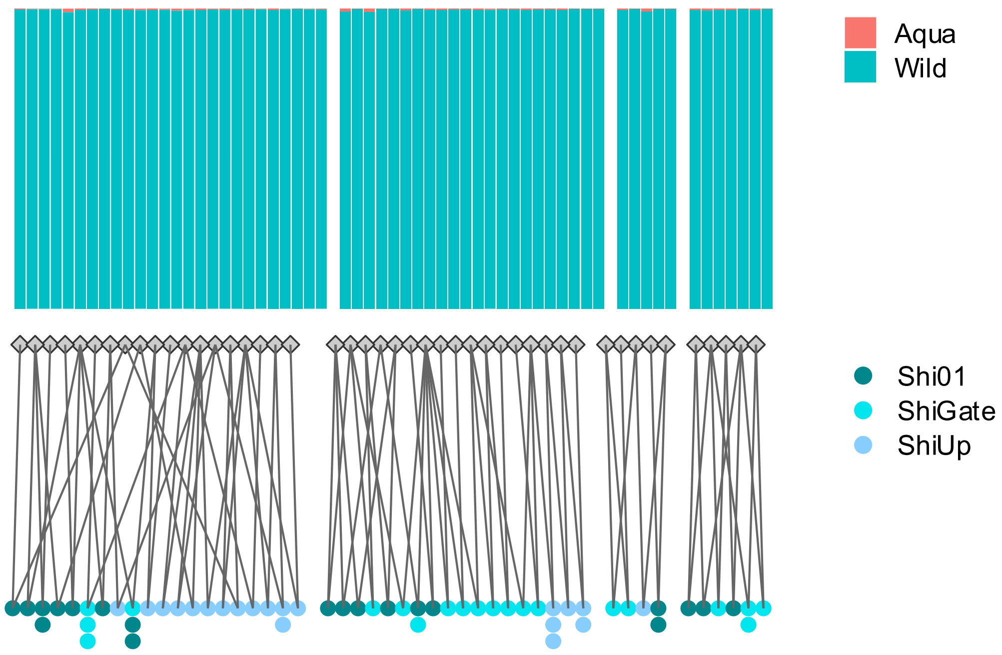
Upper Shiel 2025



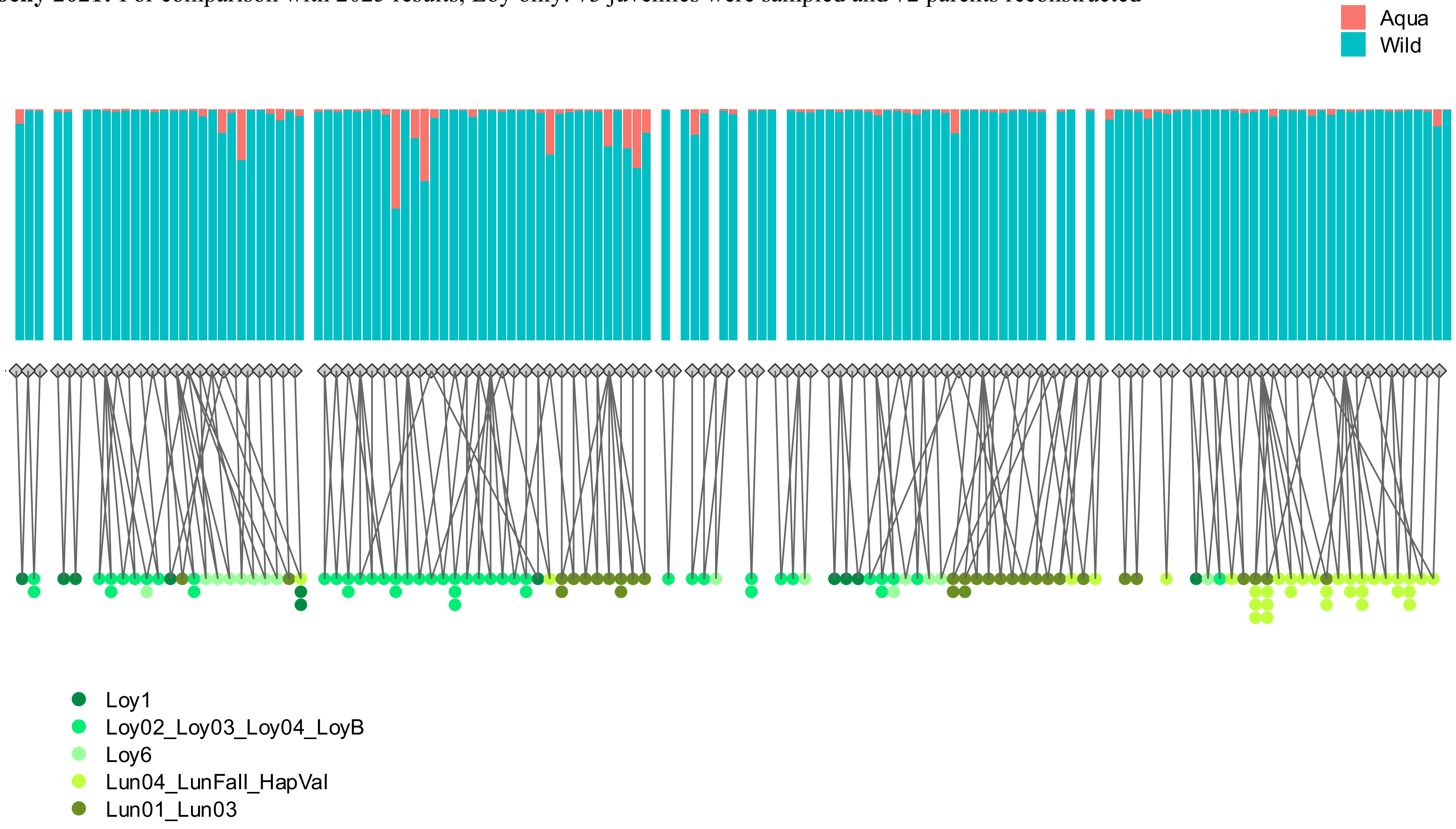

Roy 2025
Nevis 2025
Aqua
Wild
Loc35
Loc36
Loc37b
Loc39
Nev01
Nev02
Nev03
Nev04

Appendix 1: Results for previous collection years. Aquaculture introgression has been re-estimated using the same set of markers and reference fish used in the current study.

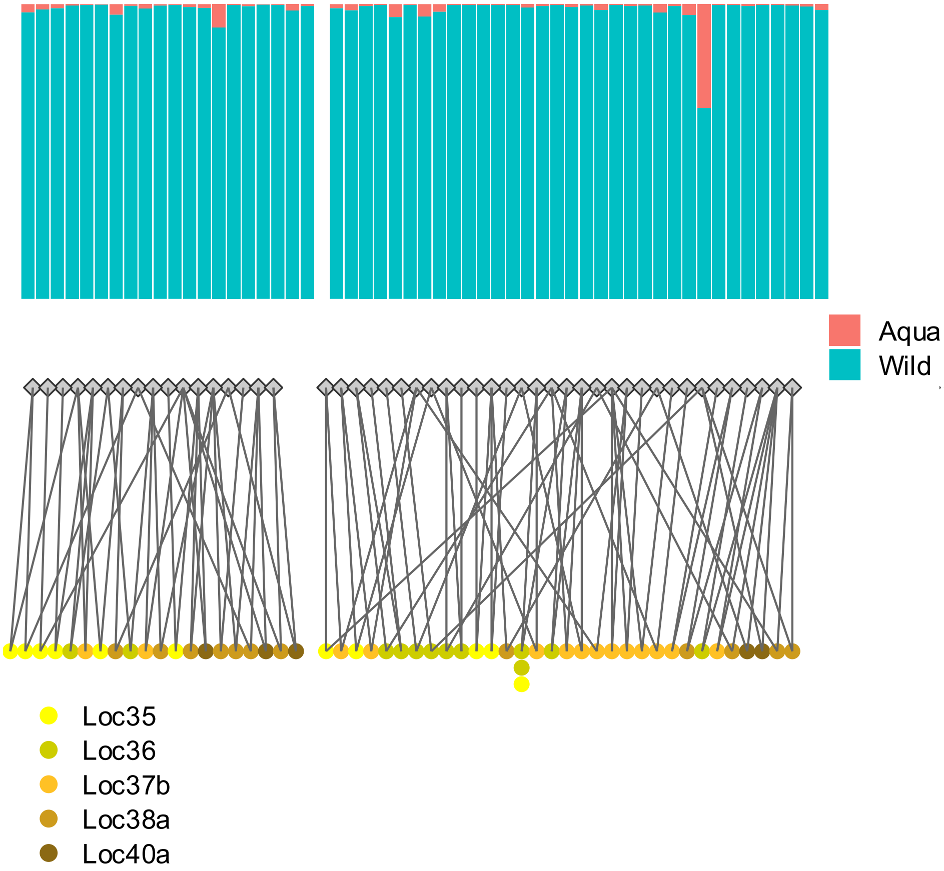
Lower Shiel 2021: For comparison with 2025 results, 60 juveniles were sampled and 46 parents reconstructed



Lochy 2021: For comparison with 2025 results, Loy only: 73 juveniles were sampled and 72 parents reconstructed



Roy 2024: For comparison with 2025 results:54 juveniles were sampled and 49 parents reconstructed



Nevis 2024: For comparison with 2025 results: 56 juveniles were sampled and 56 parents reconstructed