Project to Establish the Genetic Composition of Contemporary Wild and Farmed Atlantic Salmon Populations in the Loch Ness, Garry, Arkaig, Lochy and Shiel Catchments of Northern Scotland.

Phase 1: Genetic Baseline

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1. Introduction

Escapes of farmed Atlantic salmon (*Salmo salar*) may threaten the genetic integrity of native Atlantic salmon populations through interbreeding, with implications for their life history and fitness (e.g. Bolstad *et al*. 2021). Any genetic contribution of escaped Atlantic salmon to a wild population requires survival until maturation, followed by successful reproduction and development of the next generation. Although both survival and reproduction in the wild may be reduced in domesticated farm fish compared to wild fish, numerous studies have identified genetic changes in wild populations that can be attributed to the effect of escaped aquaculture fish or those used historically for restocking purposes (Glover *et al.* 2017).

1.1 Mowi Scotland and the ASC standards

Mowi Scotland Limited is a major operator of Atlantic salmon aquaculture in Scottish waters. A key aim of the company's Sustainability Strategy is to certify its operations in accordance with the most stringent sustainability standards which underpin environmental and social performance throughout the Mowi value chain. A part of this is a commitment from Mowi to achieve certification from the Aquaculture Stewardship Council (ASC) and in respect of production of Atlantic salmon (smolts) in pens in freshwater lochs this requires meeting the requirements of the ASC Freshwater Trout Standard[1]. The ASC Freshwater Trout Standard requires a farm to produce environmental assessment data that will demonstrate compliance with specific standard requirements relating to minimising impacts on local freshwater ecosystems and their biodiversity. One possible impact on biodiversity is the escape of juvenile Atlantic salmon (smolts) being reared in freshwater pens leading to breeding with wild stocks of salmon in the same and linked catchments. In this context, the ASC Freshwater Trout Standard requires that:

- The farm shall conduct and make public, in collaboration with the local fishery trust(s), a scientific baseline study to determine the genetic composition of the contemporary wild and farmed salmonid population(s) within the waterbody it operates in (Indicator 8.3).

- The baseline study shall include known historical farmed and wild salmonid genetic profiles and determine if changes in the genetic composition of the contemporary wild salmonid population(s) have occurred. Where changes in the genetic profile of the wild salmonid population(s) are detected, the study seeks to determine if these have occurred through introgression of farmed salmon or through other mechanisms (e.g., natural straining or stocking programmes) (Indicator 8.4).

- The baseline study will be updated every 3 years to determine if changes in the genetic composition of the wild salmonid population have occurred, and where detected, if changes are due to introgression of ASC-certified farmed smolts cultured within the same waterbody. This review shall be publicly available as well (Indicator 8.5).

Mowi Scotland has commissioned the UHI Institute for Biodiversity and Freshwater Conservation to perform such a scientific study for the catchments in Scotland that host their freshwater loch salmon farms.

This report presents results of Phase I of this project. The aim of Phase I is to establish a baseline for the genetic makeup of the wild and aquaculture-raised Atlantic salmon populations in all loch catchments hosting Mowi freshwater salmon farms. Due to a lack of suitable historical material, it is not possible to examine the genetic composition of the wild salmon populations in these catchments prior to Atlantic salmon aquaculture, which started locally over four decades ago. Therefore, the contemporary genetic makeup of the wild populations will be considered the baseline. This will provide

[1] ASC-Freshwater-Trout-Standard_v1.2_Final.pdf (asc-aqua.org)

the genetic reference point against which any future genetic changes in the populations, such as those driven by escaped aquaculture fish, can be measured. It will also provide genetic information on the domestic fish stocked into the freshwater farms that could be used to infer the source of the aquaculture fish that are driving any genetic changes. In the years following the baseline data collection, a programme of repeat sampling at the same locations will take place over three years. This will form the second, monitoring, phase (Phase II) of the study and will identify any patterns of change against the baseline.

1.2 Mowi Scotland's freshwater Atlantic salmon pen culture operations.

There are several stages involved in the production of aquaculture raised salmon. Broodstock are selected and spawned, and eggs from multiple broodstock families are transported to land-based freshwater hatcheries where they are reared to the early or mid-juvenile stage. These juveniles may then be maintained in the hatchery or transferred to open freshwater facilities, such as net pens within freshwater lakes, where they are reared on until the smolting stage. Subsequently, they are transferred to marine facilities where they are grown on until harvest. In Scotland the production eggs are received into the Mowi hatcheries (Inchmore, Inverpolly and Lochailort) from a single year class from late November through to May the following year. The fry/parr from these eggs go to freshwater loch sites from June through to January the following year. These fish are then moved to the marine locations from July through to May in the subsequent year.

Mowi Scotland rears juvenile fish in net pens at five farm sites in freshwater lochs in Scotland (Fig. 1):

Loch Ness: Loch Ness is a very large lake that receives water from a catchment area of approximately 1,800 km^2, with 180 sub-catchments flowing directly or indirectly into the loch. Mowi has cultured juvenile Atlantic salmon at one site in Loch Ness since 2014. Previous to this, a different operator cultured Atlantic salmon at the same location for approximately 20 years.

Loch Garry: Loch Garry is located in the Loch Ness catchment; water flows from Loch Garry to Loch Ness via Loch Oich. The pen culture of juvenile Atlantic salmon in Loch Garry started in 1980. There were previously three separate farming locations within the loch however these have been consolidated into a single location.

Loch Arkaig/ Loch Lochy: The Lochy and Arkaig catchments are connected to each other, with Loch Arkaig flowing into Loch Lochy. The pen culture of juvenile Atlantic salmon in Loch Arkaig started in 1985. There were previously two separate farming locations within Loch Arkaig however these have since been consolidated into a single site.

Pre-dating Mowi farming operations in Loch Lochy there were three separate operational pen fish farms rearing rainbow trout (*Oncorhynchus mykiss*). Mowi commenced rearing juvenile Atlantic salmon in 2009 at the location of one of the former rainbow trout farms and is presently the sole operator in the waterbody.

Loch Shiel is a 28 km long loch, with a total catchment area of 250 km^2 and 11 sub-catchments. Atlantic salmon have been cultured in Loch Shiel since 1978. There were previously five separate farming locations with the loch however these have since been consolidated into a single farming location ('Glenfinnan farm site')

1.3. Possible sources of aquaculture introgression into wild Atlantic salmon in the focal drainages

There are multiple possible sources of aquaculture ancestry in the wild Atlantic salmon currently present in the catchments of Lochs Ness, Garry, Arkaig, Lochy and Shiel.

1. Historic restocking initiatives*:* Information from personal communications and review of historic hatchery logbooks confirm that between 1978 and the early 1990s excess farmed salmon juveniles – a

mixture of Scottish and Norwegian genetic strains - were gifted to local fisheries owners who stocked them into a variety of wild salmon rivers. This was often a condition of lease agreements where farms had been set up on Loch systems owned by the various estates. These rivers included tributaries of Lochs Ness, Garry, Lochy, and Shiel. Such approaches, while well intended at the time, are no longer considered good practice and the available evidence indicates the practice was discontinued in the early 1990s.

2. Escapement of sea-farmed fish*:* Multiple operators raise adult Atlantic salmon in marine net pens along the west and north coast of Scotland including Orkney and Shetland. Adult fish escaping these net pens may enter freshwater and attempt to breed with the native wild salmon populations. This is frequently observed in Norway, where studies have shown a large percentage of a breeding population in a river may be escaped aquaculture fish (Glover *et al.* 2017). In Scotland, due to the geographic distribution of marine pens, catchments draining to the west and north (including Lochs Arkaig, Lochy and Shiel) may be more likely to receive escaped farm raised adult salmon than those draining to the east (including Lochs Garry and Ness). However, escaped adults may be preferentially return to the freshwater location where they were maintained as juveniles and smolts (Haraldstan *et al.* 2022).

3. Escapement of juveniles cultured in freshwater: Juveniles that escape from farms in freshwater lochs prior to smolting may enter the tributaries that flow into these lochs and mingle with the juvenile wild salmon population. They may hybridize with wild salmon at two different life stages: as 'precocious parr' before their marine migration (males only), or as adults after migrating to the marine environment and returning to the drainage where they were reared as juveniles. Within the catchments studied, farm escape events have historically been reported in Loch Garry and Loch Lochy. The latter occurred prior to the establishment of Mowi operations in the loch and involved rainbow trout, a species that does not hybridize with Atlantic salmon. While Mowi has not recorded an escape event since 2014 (Loch Garry), for the context of this study, Atlantic salmon juveniles escaping from freshwater pens in 2019-2021 could spawn as precocious parr to produce offspring that hatch in 2020-2023 and could spawn following a marine migration to produce offspring that hatch in 2021-2026.

4. Study material: Experimental trials that involve release of farm raised juvenile salmon into the wild have previously been approved by regulatory bodies. For example, the "Garry Dam Screens Project" (2017-2019) was commissioned by the generation operator to determine the survival rate of juvenile salmon passing through an unscreened turbine and tail race intake screens into the downstream River Garry. With regulatory approval, Mowi donated live juvenile salmon from its Lochailort fish hatchery to the study.

5. Subsequent hybrid generations: Once an escaped aquaculture salmon has spawned with a wild salmon, the hybrid offspring will form a part of the wild population and may survive and contribute to subsequent generations. Identification of hybrid offspring between divergent genetic groups becomes increasingly statistically difficult with increasing number of generations following the initial hybridization event (Pritchard *et al.* 2016).

2. Methods

2.1 Collection of genetic samples

Fin clip samples from putative wild juvenile Atlantic salmon were collected annually between 2018 and 2021 at a set of 23 sampling sites in the Ness, Garry, Arkaig, Lochy and Shiel catchments (Table 1a, Figure 1). Briefly, 0+ aged fry and 1+ aged parr were caught by electrofishing; each fish was anaesthetised, a small tissue sample was taken from the caudal fin; the fish was then allowed to recover and returned to its capture location. Tissue samples were preserved in pre-labelled tubes containing 70% ethanol and these were sent to the IBFC laboratory for genetic analysis. Samples were not collected

at all sampling sites at all years due to inaccessibility of sites or low numbers of juvenile salmon. Similarly, sample numbers varied between years due to fluctuations in juvenile abundances. All sampling of wild salmon was performed under the relevant licenses by the Ness District Salmon Fishery Board, the Beauly and Ness Fisheries Trust and the Lochaber Fisheries Trust.

Fin clip samples were also collected from aquaculture salmon stocked into pens in lochs Ness, Garry, Arkaig, Lochy and Shiel in 2019, 2020 and 2021. In 2019, samples were taken from fish that had already been distributed to the pens; in 2020 and 2021 the samples were taken from different batches of fish received from the hatchery prior to their distribution into the freshwater pens. Over this period, stocked juveniles represented three different aquaculture strains (Mowi Ireland, Stofnfiskur and Aquagen) and were received in various batches from three different hatcheries (Lochailort, Inchmore, Inverpolly) (Table 2).

2.2 DNA extraction and genotyping-by-sequencing

All samples were processed in 96-well plates each of which included at least one 'blank' control well containing no salmon tissue. DNA was extracted from approximately 2mm^2 of each fin clip using HotSHOT alkaline lysis (Truett *et al*. 2000). DNA concentration was measured by spectrophotometry using the QiaExpert system (Qiagen) and DNA was diluted with 10mM Tris to a standard concentration of 10ug/µl using a QIAgility liquid handling robot (Qiagen). Each sample was genotyped for a panel of 101 short tandem repeat markers ('microsatellites') as described by Bradbury *et al.* 2018. The markers were amplified in 2-4 separate multiplex PCR reactions containing the following: 3µl 2x Qiagen Type-IT multiplex master mix, 0.3µl primer multiplex mix (22-46 primer pairs at a mean concentration of 1µM per primer), 2.7µl diluted DNA. Thermocycling conditions were: 95°C for 15min, followed by 25 cycles of [94°C 30s, 57°C 3min, 72°C 30s]. with a final extension step of 72°C for 10min. The separate sets of PCR product from each sample were pooled together and diluted 40x with molecular grade water. 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], 7C for 10 min. Between 224 and 768 samples (including blanks) were pooled into a single sequencing library. Library purification and fragment size selection was then performed using Agencourt AmPure XP beads. The concentration of the pooled library was measured using a KAPA library quantification kit on the Agilent AriaMX RT-PCR system and standardized. Each pooled library was single end sequenced on an Illumina MiSeq using Illumina V3 sequencing chemistry (150 cycles). Sequence reads were demultiplexed to individual samples on the basis of their sample-specific indices and output in fastq format.

2.3 Statistical analysis

Microsatellite genotypes were called from DNA sequence reads using MEGASAT (Zhan *et al.* 2017), using an IBFC standard pipeline with a minimum sequence read depth of 20. Eight microsatellite loci were immediately removed from the dataset due to known genotyping problems in Scottish Atlantic salmon (heterozygosity deficiency, large amounts of missing data or poor genotype repeatability). Brown trout (*S. trutta*) or trout-salmon hybrids were identified from a known combination of non-amplifying loci and brown-trout specific alleles and removed. Duplicate individuals (for example wild juveniles repeatedly sampled in different years) were identified using the package rubias (Moran & Anderson 2018) in R 4.0.3 (R Core Team 2020) and consolidated. Any individual with < 15% missing data or suspected sample contamination (evidenced by the presence of loci with more than the expected two microsatellite alleles) was removed. Finally, any locus with <10% missing data was also excluded from further analysis.

The program STRUCTURE (Pritchard *et al.* 2020) was used to infer the ancestry of all sampled individuals. The following parameters were used for all STRUCTURE analyses: 100,000 burn-in followed by 200,000 MCMC steps; no a-priori information about sample origin (i.e., the program was not provided any information about an individual other than its genotype); admixture allowed; allele frequencies correlated among ancestry clusters; all other parameters default.

Initially, we applied STRUCTURE to test whether it was possible to discriminate between different genetic strains and/or juvenile batches of aquaculture fish received, which could provide further clarity on the source of any aquaculture fish or aquaculture-wild hybrids subsequently identified in the wild-sampled population. We ran STRUCTURE with the aquaculture samples only, allowed k = 1-15 possible ancestral clusters to be present in the dataset, and identified the ability of the algorithm to discriminate different groups by examining how individuals were assigned ancestry among clusters at each value of k. We considered groups (strains or batches) to be discriminated when individuals in the same group had the majority of their ancestry assigned to the same, distinct genetic cluster.

Subsequently, we used STRUCTURE to infer the presence of aquaculture ancestry in wild-sampled juveniles. We first ran STRUCTURE for multiple possible values of k ancestral clusters to identify the minimum k that would allow us to partition ancestry between wild Scottish salmon and the different genetically distinct groups of aquaculture salmon. We then examined inferred ancestry of all sampled individuals from the chosen number of ancestral clusters.

3. Results

3.1 Sample collection and genotyping quality control

IBFC received a total of 1,830 fin-clips from wild-sampled juveniles (collected 2018-2021, Table 1) and 1,218 fin-clips from aquaculture fish stocked into the five focal fish farms (collected 2019-2021, Table 2). Of these, 96 were found to have been taken from brown trout (66) or F1 trout-salmon hybrids (30, Table 3) and 22 from repeat-sampled fish. A further 89 samples from wild fish and 79 samples from aquaculture fish failed genotyping quality controls and were removed from the dataset (Table 4), leaving 1,631 wild-sampled fish and 1,131 aquaculture fish for further analysis (Table 5). Ninety microsatellite loci were retained.

3.2 Genetic differentiation among aquaculture strains and batches

STRUCTURE results demonstrated that the three aquaculture strains stocked into the smolt pens between 2019 and 2021 could clearly be discriminated on the basis of their microsatellite genotypes at the 90 loci (Figure 3). However, we were unable to resolve any further genetic structure, for example among batches of the same strain originating from different hatcheries.

3.2 Genetic differentiation between aquaculture strains and wild fish

We found that separation between hatchery strains and wild fish using our dataset was most clearly resolved when assuming five, rather than four, genetic clusters, with the three known hatchery strains forming three distinct genetic clusters and wild Atlantic salmon represented by two clusters that partly, but not completely, reflected geographic location. Runs assuming four clusters resolved two wild and two hatchery clusters but did not split Mowi strain fish from Stofnfiskur strain fish. As we were only interested in genetic mixing between the various aquaculture strains and wild fish rather than genetic substructure of the wild population, we ran the analysis at k=5 and calculated the total ‘wild’ ancestry of each sampled fish as the sum of its ancestry from each of the two wild clusters.

Figure 3 shows the estimated ancestry of each sampled aquaculture and wild juvenile from the four ancestral clusters (Mowi, Stofnfiskur, Aquagen, wild Scottish) when all individuals are included together in a single STRUCTURE analysis. Note that, using this analysis method, every individual is

assigned a very small proportion of its ancestry (<1%) to each of the ancestral groups; this is statistical noise that is not indicative of recent hybridization. Larger amounts of Scottish ancestry inferred in some of the aquaculture fish may also represent statistical uncertainty or a true contribution of Scottish or closely related lineages to the aquaculture line. Any escaped aquaculture fish from a majority Norwegian-origin genetic line other than those in the reference database is expected to be a assigned a mix of genetic ancestry, from up to all four ancestral clusters We are not able to discriminate between wild juveniles escaped aquaculture fish of purely Scottish origin.

Several juveniles sampled across multiple years from tributaries close to the fish pens corresponded genetically to pure strains of aquaculture fish held in the pens in the same year: six Mowi-strain individuals from Slatach (Shiel) in 2019 (Fig. 2e) and seven Mowi strain plus one Stofnfiskur strain individual from Goy & Rais (Lochy) in 2020 (Fig. 2d).

4. Discussion

Here, we have presented the results of a multi-year project to establish a genetic baseline of wild Atlantic salmon in the catchments that host Mowi Scotland's freshwater net-pen aquaculture operations. This will form the basis of ongoing surveillance monitoring to assess any continuing genetic impacts of Mowi freshwater farm impacts on wild salmon in the local catchments.

We observed substantially more inferred ancestry from Norwegian-origin aquaculture lines in catchments draining to the west coast (Arkaig, Lochy, Shiel) than those draining to the east coast (Ness, Garry). Inferred aquaculture ancestry was not evenly distributed across the sampling sites in these west coast catchments. In the Shiel catchment, we identified substantial aquaculture ancestry at only two of the five sites: Slatach and Finnan, both of which were relatively close to the fish farm. However, the genetic characteristics of these two sites differed. The juveniles with substantial aquaculture ancestry sampled in Finnan were found in all years and appeared to be later generation hybrids originating from various different aquaculture strains. Investigation of relatedness among Finnan juveniles (data not shown) revealed that these fish represented large full-sibling groups, meaning that the substantial aquaculture ancestry present at this site was contributed by a small number of spawning adults. In contrast, six juveniles sampled in Slatach, immediately adjacent to the farm site, in 2018 appeared to be pure Mowi-strain individuals originating from at least five different parents. We cannot discount the possibility that these are recent juvenile escapees from the pens. Similarly, eight Mowi/Stofnfiskur individuals found in 2020 at sampling sites adjacent to the Loch Lochy farm are potential escapees, and the presence of two different genetic strains stocked into the farm at different times suggests that this was not a single escape event. One of these putative escapees was repeatedly sampled in two different years, demonstrating survivorship in the wild over time. Relatively little aquaculture ancestry was inferred within wild juveniles sampled in the Ness and Garry drainages and this study we did not identify any possible juvenile escapees in these two catchments.

We note that the possible impact of escapements from freshwater aquaculture facilities on wild Atlantic salmon populations in the focal catchments is not limited to genetic introgression. Escaped Atlantic salmon, rainbow trout and other cultivated salmonids can impact native wild salmon through resource competition and predation. Additionally, in recent years (2017-2021) invasive pink salmon (*O. gorbuscha*) have been reported in Scottish east coast rivers, including the River Ness. Pink salmon originate in the Pacific and are not native to Scottish waters. The species was introduced into Russian rivers in the 1960s to support fishing activity and in recent years has spread further west and south[2].

[2] https://www.gov.scot/binaries/content/documents/govscot/publications/factsheet/2019/11/marine-scotland-topic-sheets-freshwater/documents/reporting-pink-salmon-updated-june-2019/reporting-pink-salmon-updated-june-2019/govscot%3Adocument/pink%2Bsalmon%2B2021%2B2.0.pdf

Phase I of this project has successfully established a genetic baseline for the wild Atlantic salmon in the Ness, Garry, Arkaig, Lochy and Shiel catchments. We have demonstrated that the genetic markers applied can discriminate between wild Scottish fish and multiple Norwegian-origin aquaculture strains stocked into Mowi's freshwater pens into the same catchment, as well as discriminating among the different aquaculture strains themselves. It provides a firm basis for the subsequent surveillance monitoring phase of the project.

5. References

Bolstad GH, Karlsson S, Hagen IJ, Fiske P, Urdal K, Sægrov H, Florø-Larsen B, Sollien VP, Østborg G, Diserud OH, Jensen AJ, Hindar K (2021). Introgression from farmed escapees affects the full life cycle of wild Atlantic salmon. *Science Advances* 7(52): eabj3397.

Bradbury IR, Wringe BF, Watson B, Paterson I, Horne J, Beiko R, Lehnert SJ, Clément M, Anderson EC, Jeffery NW, Duffy S (2018). Genotyping-by-sequencing of genome-wide microsatellite loci reveals fine-scale harvest composition in a coastal Atlantic salmon fishery. *Evolutionary Applications* 11: 918-930.

Glover KA, Solberg MF, McGinnity P, Hindar K, Verspoor E, Coulson MW, Hansen MM., Araki H, Skaala Ø, Svåsand T (2017) Half a century of genetic interaction between farmed and wild Atlantic salmon: status of knowledge and unanswered questions. *Fish and Fisheries* 18, 890–927 (2017).

Haraldstad T., Forseth T., Olsen EM, Haugen TO, Höglund E (2022). Empirical support for sequential imprinting during downstream migration in Atlantic salmon (*Salmo salar*) smolts. *Scientific Reports* 12, 13736 Moran BM, Anderson EC (2018). Bayesian inference from the conditional genetic stock identification model. *Canadian Journal of Fisheries and Aquatic Sciences* **76:** 551-560

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

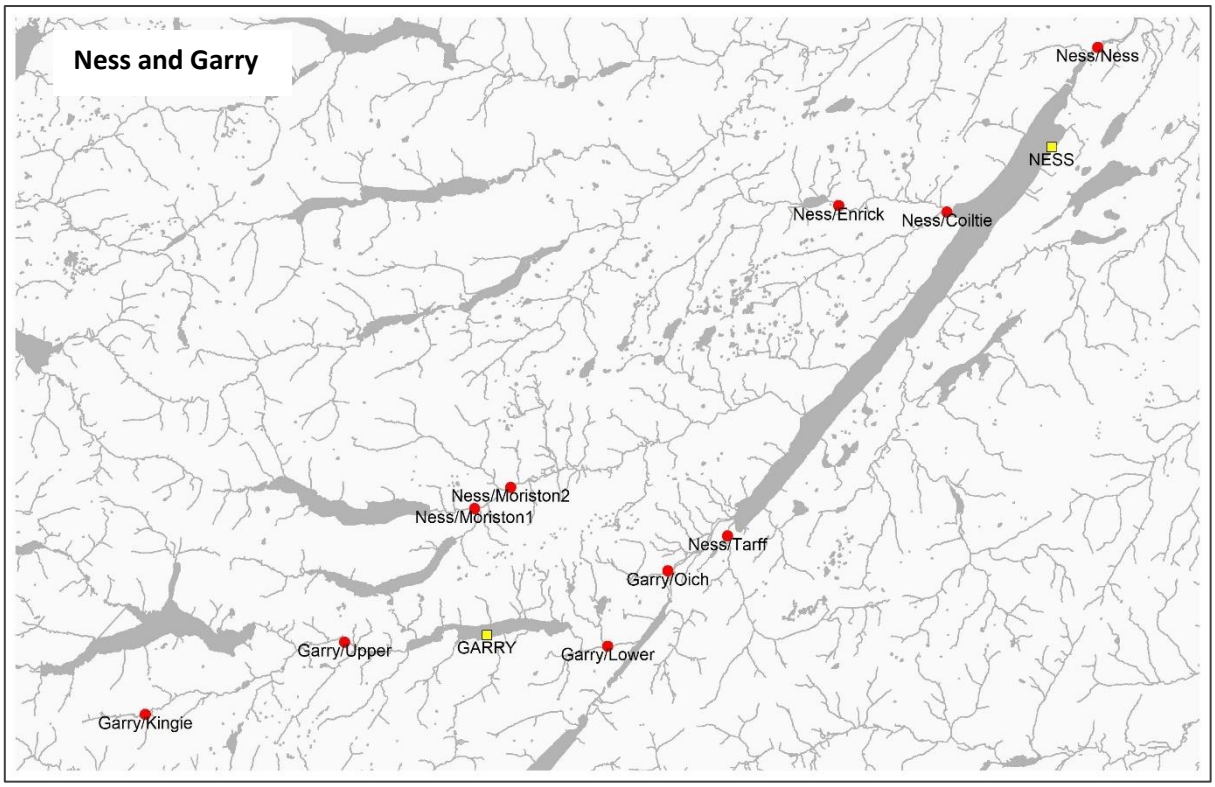
Pritchard VL**,** Orell P, Kent MP, Lien S, Niemelä E, Erkinaro J, Primmer CR (2016) SNPs to discriminate different classes of hybrid between wild salmon and aquaculture escapees. *Evolutionary Applications*, **9**, 1017-1031

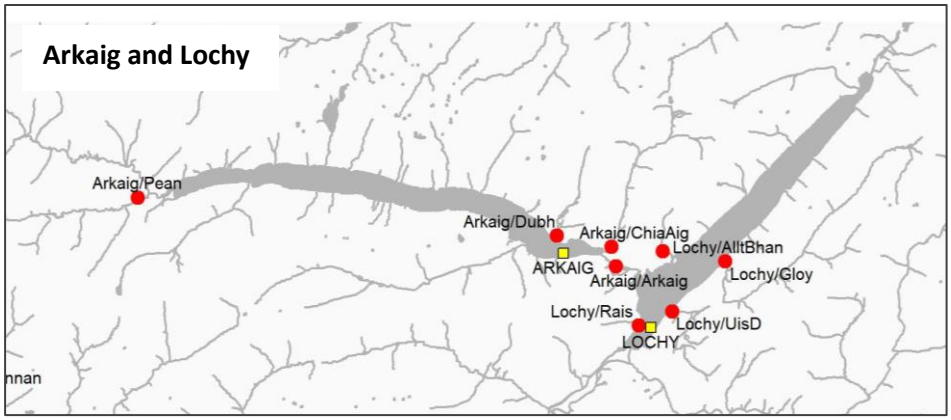
R Core Team (2020) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Truett GE, Heeger P, Mynatt RL, Truett AA, Walker JA, Warman ML. (2000) Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques* **29:**52-54.

Zhan L, Paterson IG, Fraser BA, Watson B, Bradbury IR, Nadukkalam Ravindran P, Reznick D, Beiko RG, Bentzen P. megasat: automated inference of microsatellite genotypes from sequence data. *Molecular Ecology Resources* **17:** 247-256.

Figure 1: Location of freshwater fish farms (yellow squares) and sampling sites for wild juveniles (red circles) in the Ness, Garry, Arkaig, Lochy and Shiel catchments.





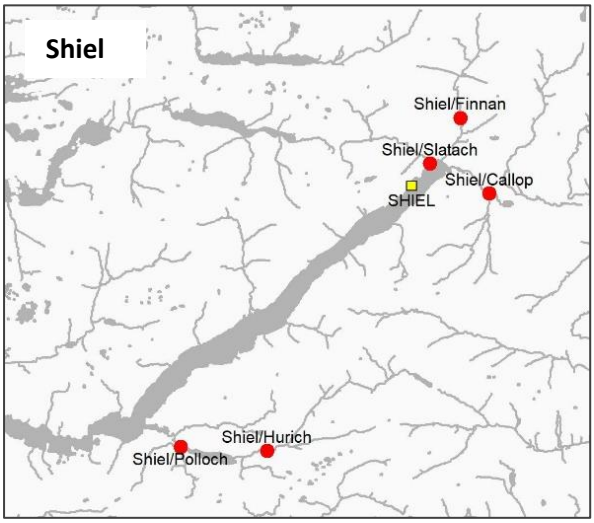


Table 1: Wild Atlantic salmon juveniles sampled at each site.

Site	2018	2019	2020	2021	*Total*
Ness/Coiltie	0	30	30	30	*90*
Ness/Enrick	40	50	31	30	*151*
Ness/Moriston1	29	21	26	12	*88*
Ness/Moriston2	36	40	30	30	*136*
Ness/Ness	35	40	30	30	*135*
Ness/Tarff	12	30	20	30	*92*
Garry/Kingie	0	34	30	30	*94*
Garry/Lower	32	30	30	30	*122*
Garry/Oich	13	30	31	30	*104*
Garry/Upper	0	3	3	4	*10*
Arkaig/Arkaig	0	27	33	27	*87*
Arkaig/ChiaAig	0	26	31	8	*65*
Arkaig/Dubh	0	18	0	0	*18*
Arkaig/Pean	0	0	4	11	*15*
Lochy/AlltBhan	0	0	26	0	*26*
Lochy/Rais	0	0	9	0	*9*
Lochy/Gloy	0	0	10	12	*22*
Lochy/UisD	0	0	15	0	*15*
Shiel/Callop	31	0	30	36	*97*
Shiel/Finnan	37	31	28	30	*126*
Shiel/Hurich	46	37	36	30	*149*
Shiel/Polloch	34	43	32	40	*149*
Shiel/Slatach	18	0	12	0	*30*
Total	***363***	***490***	***527***	***450***	***1830***

Table 2: Samples of aquaculture juveniles provided for genetic analysis

Year	Batch	Q Class	Genetics	Hatchery	When Stocked	Receiving Farm	# Samples
2019	na	Unknown	Mowi Ireland	Unknown	2019	Arkaig	30
2019	na	Unknown	Mowi Ireland	Unknown	2019	Garry	30
2019	na	Unknown	Mowi Ireland	Unknown	2019	Lochy	30
2019	na	Unknown	Mowi Ireland	Unknown	2019	Ness	30
2019	na	Unknown	Mowi Ireland	Unknown	2019	Shiel	30
2020	B1	Q4	Mowi Ireland	Lochailort	Jun 20	Lochy	75
2020	B2	Q4	Mowi Ireland	Inchmore	Jul 20	Shiel	75
2020	B3	Q4	Mowi Ireland	Inverpolly	Sept 20	Garry, Ness	75
2020	B4	Q1/Q2	Mowi Ireland	Inverpolly	Aug 20	Arkaig	75
2020	B5	Q4	Stofnfiskur	Inchmore	Aug 20	Shiel, Ness	75
2020	B6	Q1/Q2	Stofnfiskur	Lochailort	Oct 20	Lochy, Arkaig	75
2020	B7	Q2	Stofnfiskur	Inchmore	Nov 20	Shiel	75
2021	IM1	Q4	Aquagen	Inchmore	Aug 21	Ness	62
2021	IM2	Q4	Mowi Ireland	Inchmore	Sept 21	Shiel, Lochy	65
2021	IM3	Q2	Mowi Ireland	Inchmore	Dec 21	Lochy	65
2021	IV1	Q4	Mowi Ireland	Inverpolly	Jul 21	Garry	65
2021	IV2	Q2	Mowi Ireland	Inverpolly	Aug & Nov 21	Arkaig	62
2021	LA1	Q3	Aquagen	Lochailort	Jun 21	Lochy	73
2021	LA2	Q4	Mowi Ireland	Lochailort	Jul & Aug 21	Shiel, Lochy	65
2021	LA3	Q4	Mowi Ireland	Lochailort	Oct & Nov 21	Shiel	68

Table 3: Number of individuals removed from analysis due to a) being identified as trout or trout-salmon hybrids or b) failing genotyping quality control.

TROUT & HYBRIDS	2018	2019	2020	2021
Aquaculture	0	0	0	0
Ness/Coiltie	0	0	1	0
Ness/Enrick	0	0	0	1
Ness/Moriston1	0	1	2	1
Ness/Moriston2	0	0	0	0
Ness/Ness	1	1	1	0
Ness/Tarff	0	0	0	0
Garry/Kingie	0	0	0	0
Garry/Lower	0	0	0	0
Garry/Oich	0	1	5	0
Garry/Upper	0	0	0	0
Arkaig/Arkaig	0	0	7	2
Arkaig/ChiaAig	0	0	3	1
Arkaig/Dubh	0	0	0	0
Arkaig/Pean	0	0	0	7
Lochy/AlltBhan	0	0	21	0
Lochy/Rais	0	0	3	0
Lochy/Gloy	0	0	2	4
Lochy/UisD	0	0	0	0
Shiel/Callop	0	0	0	14
Shiel/Finnan	1	3	2	3
Shiel/Hurich	1	4	0	0
Shiel/Polloch	0	0	0	1
Shiel/Slatach	2	0	0	0

FAILED QC	2018	2019	2020	2021
Aquaculture	0	15	51	21
Ness/Coiltie	0	2	0	1
Ness/Enrick	1	1	2	0
Ness/Moriston1	1	0	4	0
Ness/Moriston2	2	0	0	1
Ness/Ness	1	0	0	0
Ness/Tarff	0	0	2	3
Garry/Kingie	0	2	1	0
Garry/Lower	0	0	0	0
Garry/Oich	2	0	3	0
Garry/Upper	0	0	0	0
Arkaig/Arkaig	0	5	2	3
Arkaig/ChiaAig	0	1	0	0
Arkaig/Dubh	0	3	0	0
Arkaig/Pean	0	0	0	1
Lochy/AlltBhan	0	0	0	0
Lochy/Rais	0	0	0	0
Lochy/Gloy	0	0	0	1
Lochy/UisD	0	0	0	0
Shiel/Callop	20	0	0	0
Shiel/Finnan	1	3	0	1
Shiel/Hurich	16	11	1	1
Shiel/Polloch	0	2	0	2
Shiel/Slatach	0	0	0	0

Table 4: Final number of individuals analysed

Site	2018	2019	2020	2021	*Total*
Aquaculture	0	135	474	522	*1131*
Ness/Coiltie	0	28	29	29	*86*
Ness/Enrick	39	49	29	29	*146*
Ness/Moriston1	28	20	20	11	*79*
Ness/Moriston2	34	40	30	29	*133*
Ness/Ness	33	39	29	30	*131*
Ness/Tarff	12	30	18	27	*87*
Garry/Kingie	0	32	29	30	*91*
Garry/Lower	32	30	30	30	*122*
Garry/Oich	11	29	23	30	*93*
Garry/Upper	0	3	3	4	*10*
Arkaig/Arkaig	0	22	24	22	*68*
Arkaig/ChiaAig	0	25	28	7	*60*
Arkaig/Dubh	0	15	0	0	*15*
Arkaig/Pean	0	0	4	3	*7*
Lochy/AlltBhan	0	0	5	0	*5*
Lochy/Fairy	0	0	6	0	*6*
Lochy/Gloy	0	0	8	7	*15*
Lochy/UisD	0	0	15	0	*15*
Shiel/Callop	11	0	30	22	*63*
Shiel/Finnan	35	25	26	26	*112*
Shiel/Hurich	29	22	35	29	*115*
Shiel/Polloch	34	41	32	37	*144*
Shiel/Slatach	16	0	12	0	*28*
Total	*314*	*585*	*939*	*924*	*2762*

Figure 2a: Inferred ancestry of aquaculture fish when aquaculture and wild juveniles are analysed together. Each column represents an individual fish, with colour indicating different ancestral clusters.

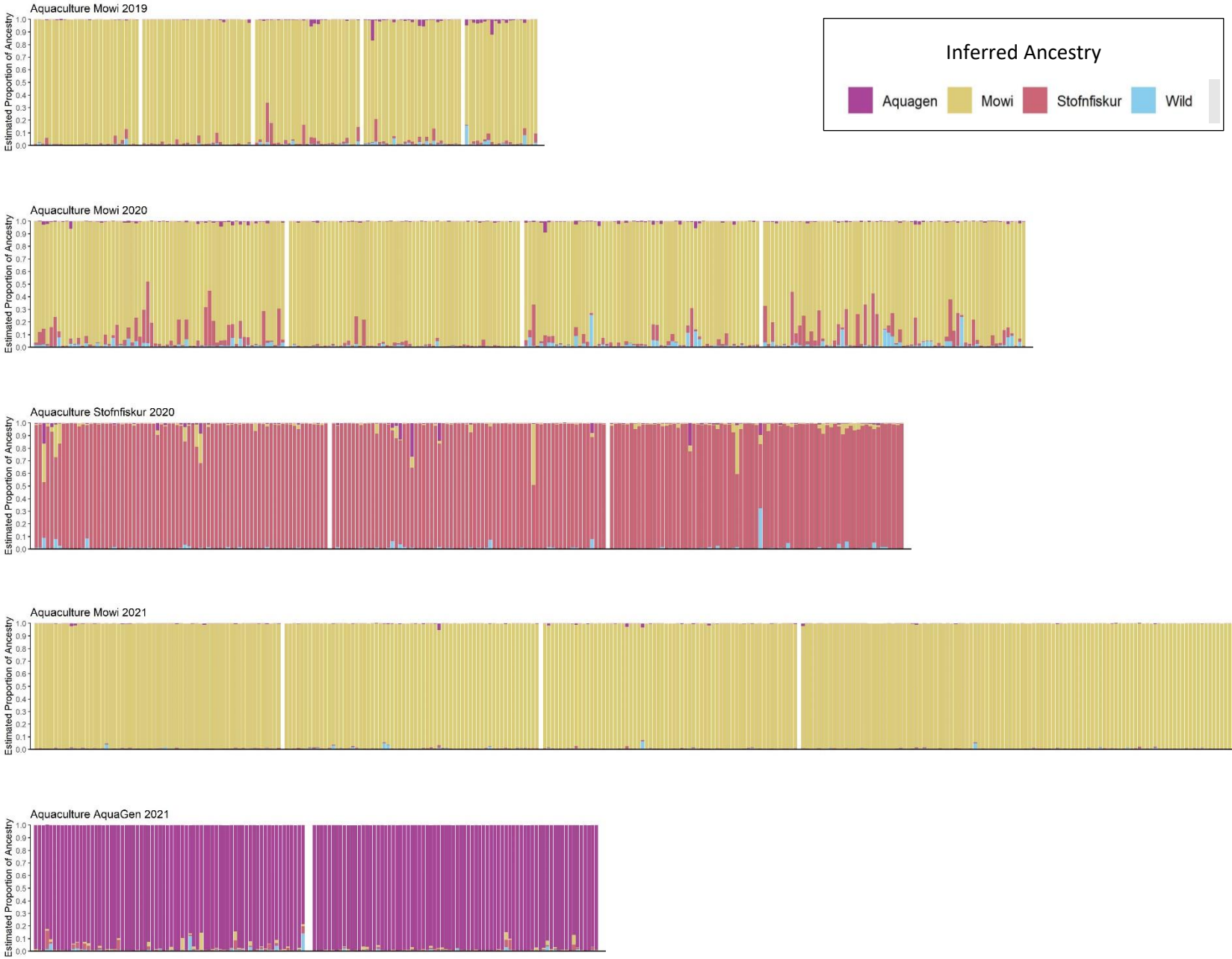


Figure 2b: Inferred ancestry of each genotyped fish from three aquaculture strains and wild Scottish fish, Ness drainage. Each column represents an individual fish, with colour indicating different ancestral clusters. The strain stocked in the fish farm is shown for each year.

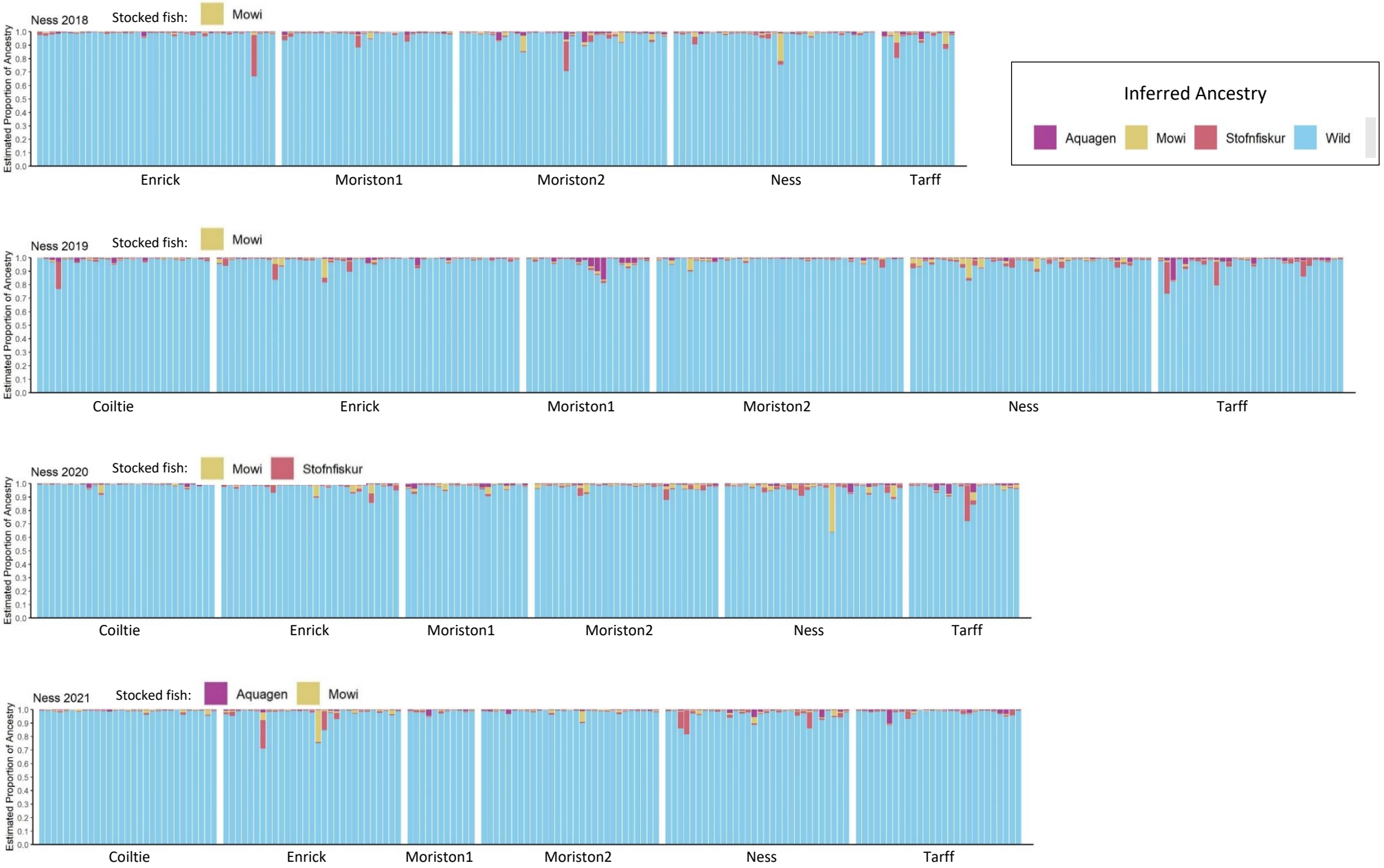


Figure 2c: Inferred ancestry of each genotyped fish from three aquaculture strains and wild Scottish fish, Garry drainage. Each column represents an individual fish, with colour indicating different ancestral clusters. The strain stocked in the fish farm is shown for each year.

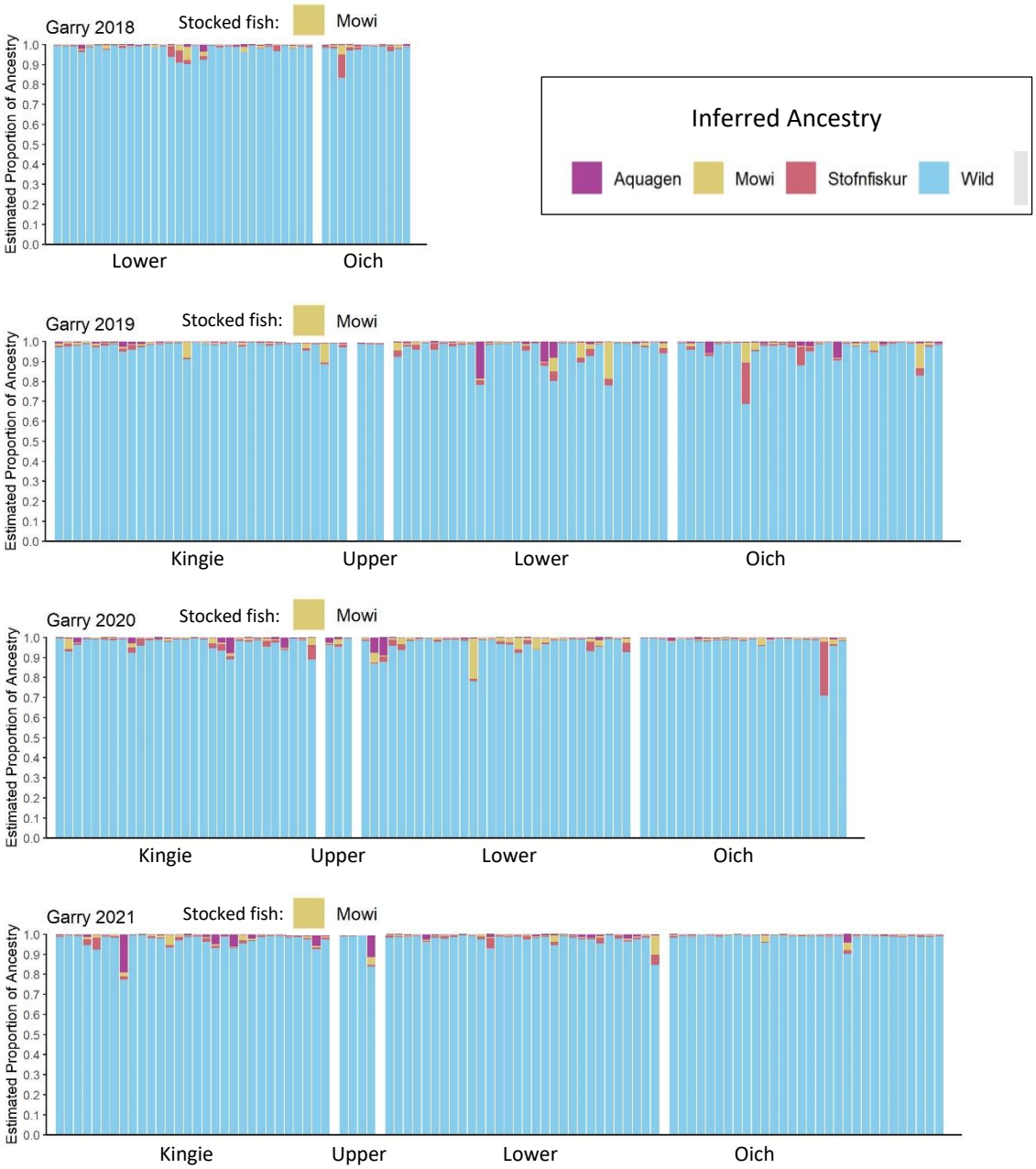


Figure 2d: Inferred ancestry of each genotyped fish from three aquaculture strains and wild Scottish fish, Arkaig and Lochy drainages. Each column represents an individual fish, with colour indicating different ancestral clusters. The strain stocked in the fish farm is shown for each year.

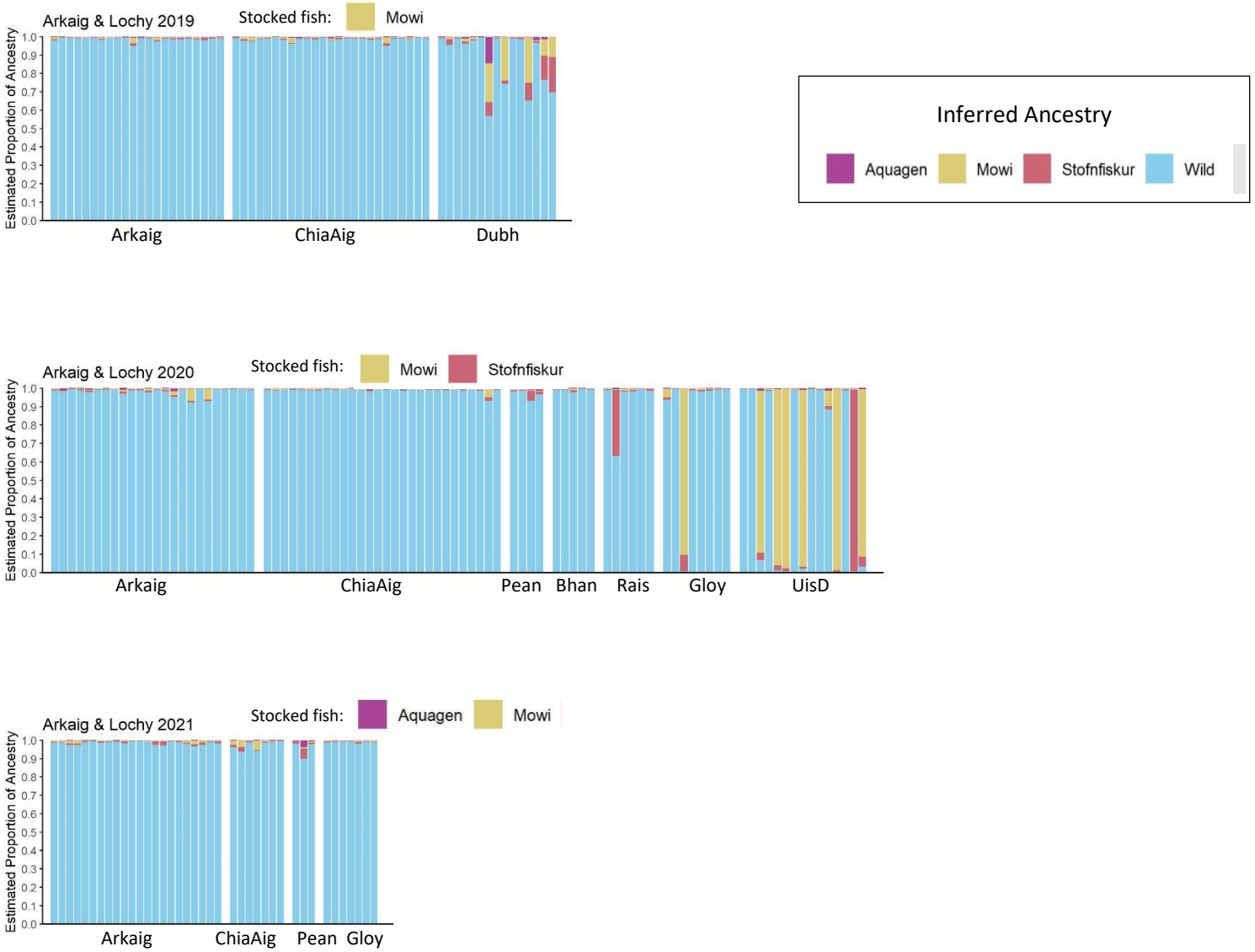


Figure 2e: Inferred ancestry of each genotyped fish from three aquaculture strains and wild Scottish fish, Shiel drainage. Each column represents an individual fish, with colour indicating different ancestral clusters. The strain stocked in the fish farm is shown for each year.