River Carron Catchment Scale Restoration Programme Phase IIa:

Assessment of the Proportion of Offspring from the 2014 Carron Atlantic Salmon Broodstock among Smolts and Returning Adults.

Rivers & Lochs Institute, Inverness College, University of the Highlands and Islands.

Project Lead: Victoria Pritchard

Project Support: Silvia Ferreira Carvalho, Barbara Morrissey, Dasha Svobodova

Project Development: Eric Verspoor, Melanie Smith, River Carron Conservation Association

Background

Supplementary stocking - producing large numbers of offspring from broodstock in a captive hatchery and releasing them into the natural environment - has long been a tool in the management of salmon populations. Historically used to increase the number of adult fish available for harvest, more recently it has been applied with the aim of mitigating for human impacts that have increased population extinction risk. For example, supplementary stocking is believed to have aided in the early recovery of the River Tyne Atlantic salmon stock (Milner *et al.* 2004) and has been demonstrated to increase numbers of wild-spawning adults in other systems (Hess *et al.* 2012, Berejikian *et al.* 2008). Captive salmon broodstocks are maintained as 'gene banks' to preserve populations at danger of extinction in the wild (O'Reilly & Doyle 2007), and carefully managed stocking programmes could be used to maintain the genetic diversity of declining wild populations (Hedrick *et al.* 2000).

Recently, however, supplementary stocking has become less accepted as a management action for wild salmon. This is because of mounting scientific evidence that, in many cases, it may not benefit the target population and has potential to cause harm. Captive breeding and rearing, particularly over multiple generations, is expected to select for fish that are better adapted to the captive environment than to the wild (Araki *et al.* 2007, Milot *et al.* 2018), and this might be accelerated if genes from escaped domesticated salmon are accidentally introduced into a broodstock (Hagen *et al.* 2019). Reflecting this, and other hatchery effects, salmon stocked as juveniles are less likely to return as adults, and produce fewer offspring compared to their wild compatriots (Jonsson *et al*. 2003; Christie *et al.* 2014, Milot *et al.* 2018). At the same time, introduction of hatchery-spawned juveniles may reduce the survivorship of wild-spawned fry and parr (Peery *et al.* 2004, Imre *et al.* 2005), meaning that stocked fish may constitute a high proportion of adult returners despite their overall lower survivorship. Removal of wild adults for use as broodstock parents also reduces the total wild breeding population. For these reasons, supplementary stocking could even reduce the number of adults returning to the river in comparison to a no-stocking scenario (O'Sullivan *et al.* 2020). Correspondingly, some large Atlantic salmon supplementary stocking programs have been found to have minimal effects on population recovery (e.g. Griffiths *et al.* 2011; Romakkaniemi 2008). Simultaneously, because broodstock are often founded from, and maintained with, relatively small numbers of breeding adults they are often less genetically diverse then the recipient populations (Wang & Ryman 2002). Supplementary stocking can therefore both introduce hatchery-adapted genes and reduce the overall genetic diversity of the wild population, which may reduce the probability of wild population persistence after a stocking programme is discontinued.

While it is now accepted that supplementary stocking programmes can have negative effects on wild salmon, these do not necessarily outweigh the potential positive effects on populations threatened with extinction (Naish *et al*. 2007). The balance of threats and benefits is likely to be population specific and depend on how the stocking programme is managed. To date, relatively few supplementary stocking programmes have been evaluated to assess the contribution of hatchery

released fish and subsequent genetic effects in the wild populations (Kitada 2018). Of the studies that have been done, some have demonstrated no reduction of genetic variation despite multiple generations of supplementary stocking (Heggnes *et al.* 2006, Hess *et al.* 2012), while others have shown negative genetic effects (Hagen *et al.* 2021). More of these studies are needed.

The River Carron Atlantic salmon stocking programme

The River Carron on the west coast of Scotland is 17km long and prone to spate flooding. This flooding has occurred frequently in recent years consistent with the increased frequency of high intensity rainfall events predicted under climate change (Chan *et al.* 2018). The river supports a breeding population of Atlantic salmon. Salmon catches declined from a high of 200+ fish in the mid 1970s-80s to a 1996-2001 average rod catch of 10.3 fish per year. The reasons of this progressive decline are uncertain: redd washouts by concurrent spates has been proposed as a cause, however the decline was mirrored in salmon rivers throughout Scotland. Because the declining salmon population was considered at risk of extinction, the Carron riparian owners (collectively the River Carron Conservation Association, RCCA) instituted a complete catch-and-release policy and initiated a supplementary stocking programme. Due to the low numbers of adults, it was considered necessary to develop a captive broodstock; this was started in 1995 using gametes from seven wild-caught females. The broodstock has been maintained to the present day, supplemented annually by returning adults from the Carron rod catch. Since 2000, up to several hundred thousand hatchery-produced juveniles (eggs fry, parr and smolts) have been stocked into the Carron each year. Following the initiation of this supplementary stocking programme, the Carron Atlantic salmon population rebounded. Recorded catches are now higher than those at the previous peak in the 80s/90s. The continued spate flooding of the Carron, and the low numbers of salmon returning to adjacent west-coast rivers, raises the question of whether wild salmon would have persisted in or naturally re-colonized the Carron without this intervention. It is not known whether the observed increase in numbers of returning adults is a result of the stocking programme, nor whether the stocking has changed the characteristics of the Carron Atlantic salmon population in other ways.

The Carron salmon population and the supplementary stocking programme has been monitored by various methods over the past 25 years, including detailed cross and stocking records, enumeration of juveniles, smolts and adults, and coded wire tagging of stocked juveniles to monitor survivorship to adulthood. The Rivers and Lochs Institute previously partnered with the Carron riparian owners in Phase I of the River Carron Restoration Programme, which used monitoring data collected up to 2014 to assess the reasons for the population decline and rebound. Results of this project showed a strong correlation between the number of juveniles stocked and the number of adults returning to the river. While demographic modelling showed that a similar population rebound could have been possible with natural spawning alone (Curran *et al.* 2014), the RCCA consider it unlikely that sufficient wild adults were returning or straying into the Carron to enable such a natural rebound. Since 2011, tissue samples have been collected annually from the broodstock parents, as well as from stocked juveniles and fish caught at various life stages in the river by electrofishing, smolt trapping and angling. This enables the use of genetic tools to assess the impact of the supplementary stocking programme on the Carron population. By using suitable genetic markers and having samples from all broodstock parents, stocked fish can be discriminated from wild-spawned fish by matching them to their parents, a method know as ‘parentage-based tagging’ (PBT). This can be used in the same way as physical tagging of juveniles to investigate such questions as the relative survival to adulthood of stocked and wild fry. The advantages of PBT over physical tagging include lower costs (there is no need to tag large numbers of juvenile fish, most of which will not survive to adulthood), reduced physical burden to fish, and larger sample sizes (all stocked juveniles are ‘tagged’ by their unique genotypes). The genetic markers applied for PBT can simultaneously be used to monitor trends in genetic diversity of

the broodstock and wild populations and potentially investigate the presence of genetic material from escaped aquaculture fish.

Study Objectives

The proposed Phase II of the River Carron Restoration Programme will combine the above information with suitable statistical and modelling approaches to ask i) whether the observed increase in adult returns is the direct result of the stocking programme; ii) whether the stocking programme has altered the genetic composition of the Carron Atlantic salmon population; and iii) whether certain stocking strategies can be recommended over others in terms of population abundance and genetic health. The ultimate aim is to integrate the results of the Phase II study with other information sources to develop an adaptive framework for future management of the River Carron that conserves its native wild Atlantic salmon population.

This report presents the results of Phase IIa, an examination of how stocked fish from a single year of broodstock crossings (2014) have contributed to the returning adult catch.

Samples

RLI was provided with the following tissue samples by the River Carron Conservation Association (Table 1): fin clips from broodstock used for brood pairings in 2014; fin clips from smolts collected in the River Carron smolt trap in 2016 and 2017, and fin clips from rod-caught adults that returned to the river in 2017, 2018 and 2019. Three separate sets of breeders were used to produce stocked juveniles in 2014: 'Attadale' – females from the captive Carron broodstock kept at Attadale, plus wild-caught males crossed with these females; 'Uist', captive Carron broodstock kept at North Uist; and 'Wild' – adults returning to the Carron that had been caught and retained for breeding. Tissue samples were either stored in tubes in ethanol (Attadale & Wild broodstock, smolts, 2017-19 returning adults) or frozen (Uist broodstock, 2018-19 returning adults). Ethanol samples had pre-existing RLI sample numbers and frozen samples were assigned RLI sample numbers on receipt. RLI was also provided with the following sample metadata: Attadale and Wild broodstock – fish sex and recorded crosses; Uist broodstock – fish sex for half of the individuals (sex was not available for the rest because labels had become detached from samples); frozen adult samples - all recorded data, which could include sex, weight, catch date and location; adult samples in ethanol - catch year and whether the individual was retained as a brood fish; smolt samples in ethanol - catch year and likely smolt age (1yr / 2+ yrs). RCCA subsequently provided additional metadata about individuals that were found to be sampled more than once (see below), and Uist crosses.

DNA extraction and genotyping

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). DNA concentration was measured by spectrophotometry using the QiaExpert system and diluted with 10mM Tris to a standard concentration of 10ug/µl using a QIAgility liquid handling robot. For each sample, 20-31 microsatellite loci (total 101 loci, Bradbury *et al.* 2018) were amplified in four separate multiplex PCR reactions containing the following: 1.75µl 2x Qiagen Type-IT multiplex master mix, 0.35µl primer multiplex mix at a concentration of 1µM per primer, 1.4µl diluted DNA. Thermocycling conditions were: 95°C for 15min, 25x [94°C 30s, 57°C 3min, 72°C 30s], 72°C for 10min. The four sets of PCR products were pooled for each sample and diluted 40x with water. Three 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 of the 288 samples (including 9 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. Product for all 288 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 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), with sequence reads demultiplexed to individual samples on the basis of their sample-specific indices and output in fastq format.

Statistical analysis

The program MEGASAT (Zhan *et al.* 2017) with user-provided input, was used to call genotype at each of the 101 targeted microsatellite loci for each individual based on the DNA sequence information in the fastq file. Nine microsatellite loci were immediately removed from the dataset on the basis of known previous poor performance (low genotyping success, poor repeatability of genotype scoring or other problems). Samples with missing genotypes at > 30% of the remaining 92 loci were excluded from the dataset on the basis of having too much missing data; each of these initially excluded samples was run through the complete genotyping process a second time with the aim of improving genotyping success. 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 identify genetically identical samples (i.e those taken from the same individual fish), and these were combined into a single sample. A further six microsatellite loci were removed from the final dataset due to having >30% missing data over all individuals, leaving 86 loci to be taken forward to statistical analysis.

Genetic diversity and inbreeding: we estimated expected heterozygosity (H_e) and allelic richness (R) rarefied to the smallest sample size (both measures of genetic diversity) and F_{is} (an estimator of inbreeding) using the hierfstat package in R (Goudet 2005). We calculated these indices for seven different groups: 2014 Attadale, Uist, and Wild broodstock; the identified offspring of these three different broodstocks (smolts and returning adults); and all other smolts and returning adults.

Parentage reconstruction: the software COLONY 2.0.6.6 (Jones & Wang 2010) was used to identify smolts or returning adults that were the offspring of genotyped 2014 broodstock fish. The following parameters were applied: probability of allele drop out 0.05 and other errors 0.01 for all loci; allele frequency not updated; diecious parents; polygamy for both sexes; full sibship not scaled; weak sibship prior with an average maternal and paternal sibship size of 3; unknown population allele frequency; combined pairwise likelihood and full likelihood (FLPS) algorithm with medium run length and medium precision. Because sex information had been lost for half of the Uist broodstock samples, a pilot Colony run was performed with only the Uist samples as potential parents and each one allowed to be either mother or a father by specifying parents as monoecious. Information about parental combinations from this pilot analysis was used to infer the sex of the individuals with missing sex information, allowing them to be included in the correct single-sex parent group for the full analysis. Because the Carron Atlantic salmon population is small and has been intensively stocked, it is possible that a large amount of inbreeding is present in the population, which can affect the inference of offspring-parent relationships. Therefore, we ran four repeat Colony analyses: two allowing inbreeding to be present in the population, and two not allowing inbreeding to be present, and compared the results.

COLONY was also used to estimate sibling relationships among the 2014 broodstock individuals, using the same parameters as above but with no sibship prior and no inbreeding.

Genetic material from aquaculture fish: the presence of genetic material from Norwegian-ancestry aquaculture fish (the predominant type used throughout Scotland) was assessed using the program STRUCTURE (Pritchard *et al.* 2000), which can be used to infer the ancestral contribution of different genetically distinct groups to a focal individual. To do this, we combined the Carron genetic dataset with equivalent genetic datasets from two other groups: 1) several different broodstock lines of Norwegian aquaculture salmon, and 2) wild parr collected from the Arkaig, Shiel, and Ness River systems. We ran STRUCTURE specifying two ancestral groups with the following parameters: admixture model with correlated allele frequencies, no prior population information, 20,000 burn-in followed by 50,000 MCMC reps; all other parameters default. 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). For the purposes of this study, therefore, we considered any fish with an estimated mean Norwegian ancestry > 10% to contain genetic material from escaped aquaculture fish.

Results

Genotyping success: Table 1 shows the number of Atlantic salmon tissue samples put through the genotyping process, the number excluded at different quality control steps, and the number taken forward to statistical analysis. Ninety-eight percent of broodstock samples, 96% of returning adult samples and 100% of smolt samples were considered to have been successfully genotyped (i.e. < 30% missing data). The presence of genetically duplicate samples in the rod catch collections showed that 10.7% of returning adults were caught and/or fin-clipped more than once (Table 2).

Genetic diversity and inbreeding: Estimators of genetic diversity and inbreeding are provided in Table 3.

Parentage reconstruction*:* the four replicate COLONY analyses relating 2014 broodstock parents to fish sampled in the river between 2016-2020 gave nearly identical results, independent of whether or not inbreeding was specified as present. The 560 smolts and returning adults analysed were estimated to be the offspring of 486-490 distinct parents, of which 25 were genotyped 2014 broodstock males and 80-81 were genotyped 2014 broodstock females. Of these 560 fish, 90 (16%) had two parents identified among the 2014 broodstock, always from the same broodstock group (Attadale, Uist, or Wild) and nearly always a recorded cross. Depending on analysis replicate, a further 68-72 smolts/adults had a mother but not a father identified among the 2014 broodstock, and a single adult had a father identified among the broodstock but not a mother (Table 4). Reconstructed pedigrees of fish with at least one identified broodstock parent are shown in Fig, 1, and inferred parent-offspring pairs are provided in Appendix 1. Twenty six of the 133 returning adults with at least one 2014 broodstock parent were retained as brood fish in 2017-2019.

Estimated relatedness of broodstock parents: as might be expected for a captive brood line, the Attadale and Uist broodstocks included a large number of closely related individuals (Fig. 2). However, the crossing scheme for these stocks avoided sibship matings, with only three recorded or identified 2014 crosses involving inferred full-sibs (Attadale ICB868 & ICB869 x ICB870).

Genetic material from aquaculture fish: results of the STRUCTURE analysis indicated that several 2104 broodstock parents, smolts and returning adults had recent ancestry from Norwegian aquaculture fish (Figs 1-3). There was no clear difference in levels of aquaculture ancestry between the smolts and adults that were the offspring of the 2014 broodstock and the smolts and adults that were not (Fig. 3).

Discussion

Our results support the conclusions of previous physical tagging studies showing that fish stocked into the Carron as juveniles survive to return as adults. Considering fish with both broodstock parents identified, 35.6% of 2017 2+ year old smolts - most of which are expected to have been spawned in 2014 - were stocked fish. Of the adult returners in 2017-2019, up to 27.7% per year originated from the 2014 broodstock - as expected from the most common life history of 2yr smolting followed by 1yr at sea the highest proportion was seen in 2018. Since adult returners in any one year include fish spawned in three or more years, total contribution of stocked fish to the adult catch can only be estimated by genotyping additional years of broodstock; however, fish stocked in the Carron clearly survive to adulthood and make a substantial contribution to the fishery. We observed no major differences in measures of genetic diversity and inbreeding between the three broodstocks, their offspring, and other fish trapped in the river; thus, there is no indication that the 2014 stocking presented a risk to the genetic diversity of Carron Atlantic salmon as a whole.

Numerous smolts and returning adults had only one identified parent in the 2014 broodstock. A particularly large number of individuals were assigned a mother, but not a father, from the genotyped Attadale broodstock. This is not unexpected, as a substantial proportion of Attadale females used as brood parents in 2014 were also used, crossed with different males, in 2013 and 2015; thus these 'single-parent' fish are likely derived from non-2014 Attadale crosses. The 2014 Uist broodstock parents were used only in that year and therefore four missing Uist parents are likely to be individuals that were not sampled (e.g. two containers in the Uist collection did not contain fin clips) or failed genotyping quality control (Table 1). Wild broodstock parents were caught in the river and returned to it after stripping, so additional wild spawnings are another potential source of missing parents in this broodstock category.

Based on our working definition, genetic material from Norwegian aquaculture fish was identified in varying amounts in the broodstock parents, the smolts, and the returning adults. Although we did not observe any elevated aquaculture ancestry in the 2014 broodstock offspring compared to the population as a whole, we note that many of the other smolts and returning adults tested are likely to be stocked fish from other years. Therefore, at this time we cannot draw any conclusions about whether the supplementary stocking is maintaining or increasing the amount of Norwegian aquaculture material in the wild population.

Future work

These results from Phase IIa of the River Carron Restoration demonstrate how parentage-based tagging using genetic markers can both reveal the fate of juveniles stocked in the Carron and examine how the stocking programme may be impacting the wild salmon population in terms of genetic diversity and genetic material from aquaculture escapees. Thus far we have only examined a single broodstock year and focused on the returning adult life stage. A more complete assessment how the stocking programme is contributing to the health and maintenance of the Carron Atlantic salmon population requires using this approach to follow the fate of stocked vs. wild spawned fish over multiple years and life history stages and combining this with appropriate statistical analysis and mathematical modelling. Results of such an analysis will provide a firm basis for future management of the Carron to maintain the security and integrity of the wild fishery.

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Table 1: Genotyping success and quality control.

	# samples genotyped	Removed			# samples analysed
		Trout or hybrids	> 30% missing	Duplicates	
2014 Broodstock - Attadale	87	1	0	0	86
2014 Broodstock - Uist	44	0	3	1	40
2014 Broodstock - Wild	58	0	1	1	56
2016 1 Year Old Smolts	15	0	0	0	15
2017 2+ Year Old Smolts	59	0	0	0	59
2017 Adult Returners	121	1	2	15	103
2018 Adult Returners	193	2	5	20	166
2019 Adult Returners	256	2	14	23	217
Total	**833**	**6**	**25**	**60**	**742**

Table 3: Expected heterozygosity (He), alleleic richness (R) corrected for a minimum sample size of 40 individuals; and Wright's inbreeding coefficient (Fis, ranges from -1 to +1, with larger values indicating more inbreeding).

Category	n	He	Fis	R
Attadale 2014 Broodstock	86	0.558	-0.018	4.482
Uist 2014 Broodstock	40	0.522	-0.043	3.889
Wild 2014 Broodstock	56	0.577	-0.002	4.921
Attadale 2014 Offspring	72	0.551	-0.025	4.390
Uist 2014 Offspring	45	0.503	-0.087	3.734
Wild 2014 Offspring	44	0.564	0.001	4.676
Other fish	400	0.582	0.009	4.874

Table 2: Returning adults caught and/or sampled more than once.

Fish_ID	Catch1_date	Catch1_notes	Catch2_date	Catch2_notes	Catch3_date	Catch3_notes
ICI970_ICJ024_ICJ037	31/07/2017	3.5lb cock, Seat Pool	2017	Grilse cock, broodstock	2017	Grilse cock, broodstock
ICE641_ICJ025_ICJ038	2017	12lb cock, Rock Pool	2017	Salmon cock, broodstock	2017	Salmon cock, broodstock
ICE635_ICE642	06/06/2017	12lb hen, Rock Pool	08/06/2017	10lb hen, Rock Pool	na	na
ICE639_ICE645	07/06/2017	12.5lb hen, Cruives Pool	14/06/17	12.5lb hen, Big Ann's	na	na
ICE640_ICJ034	07/06/2017	9.5lb hen, Rock Pool	2017	Salmon hen, broodstock	na	na
ICE643_ICI980	09/06/2017	9lb cock, McClean's Pool	31/07/2017	8lb, Below Chisholm's Pool	na	na
ICE644_ICJ046	10/06/2017	12lb hen, House Pool	2017	Grlise hen, broodstock	na	na
ICI959_ICJ040	06/07/17	11lb hen, Bridge Pool	2017	Salmon hen, broodstock	na	na
ICI979_ICJ020	31/07/17	7lb, Chisholm's Pool	2017	Salmon hen, broodstock	na	na
ICI993_ICI996	18/08/2017	6lb hen, Brabourne's Pool	25/08/2017	5.5lb hen, House Pool	na	na
ICJ017_ICJ029	2017	Salmon cock, broodstock	2017	Salmon cock, broodstock	na	na
ICJ026_ICJ039	2017	Salmon cock, broodstock	2017	Salmon cock, broodstock	na	na
ICJ043_ICJ047	2017	Salmon cock, broodstock	2017	Salmon cock, broodstock	na	na
ICJ059_ICJ132_ICO005	19/06/2018	10lb hen, Rock Pool	12/09/2018	6lb hen, Bridge Pool	2018	7lb hen, broodstock
ICJ063_ICY727f	22/06/2018	6lb hen, Railway Bridge	2018	6lb hen, Railway Bridge	na	na
ICJ067_ICJ090	21/07/2018	6lb, Bridge Pool	09/08/2018	5lb cock, below Seat Pool	na	na
ICJ073_ICO029	02/08/2018	3lb, Big Ann's	2018	6lb cock, broodstock	na	na
ICJ077_ICJ133	04/08/2018	6lb, Rock Pool	13/09/2018	5lb cock, Whin Pool	na	na
ICJ078_ICJ128	04/08/2018	4lb, Big Ann's	31/08/2018	3lb hen, King Run	na	na
ICJ080_ICJ123	28/07/2018	4lb McNair's	28/08/2018	4lb cock, McNair's	na	na
ICJ089_ICY727c	09/08/2018	5lb hen, General's Pool	02/10/2018	5lb hen, Power Lines	na	na
ICJ098_ICJ122	07/08/2019	6lb, House Pool	28/08/2019	6lb, House Pool	na	na
ICJ103_ICJ120	20/08/2018	3.5lb hen, House Pool	23/08/2018	3lb, House Pool	na	na
ICJ107_ICY727b	21/08/2018	4lb hen, Rock Pool	04/10/2018	3lb cock, Rickety Bridge	na	na
ICJ110_ICO027	23/08/2018	10lb hen, Big Ann's	2018	10lb hen, broodstock	na	na
ICJ114_ICO016	25/08/2018	11lb hen, Big Ann's	2018	10lb hen, broodstock	na	na

ICJ130_ICO011	10/09/2018	12lb cock, McNair's	2018	12lb cock, broodstock	na	na
ICJ137_ICO014	14/09/2018	5lb hen, above Rickety Bridge	2018	4lb hen, broodstock	na	na
ICO004_ICY702	2018	7lb hen, broodstock	18/02/2019	6lb hen, kelt, Cruives	na	na
ICO023_ICY700	2018	12lb hen, broodstock	27/02/2019	15lb hen, kelt Big Ann's	na	na
ICO063_ICY514_ICY690	2019	10lb hen, broodstock	06/06/2019	12lb hen, Rock pool	01/02/2020	15lb hen kelt Big Anns
ICO055_ICY509	2019	7lb cock, broodstock	01/02/2020	4lb cock, kelt, Cruives	na	na
ICO065_ICY532	2019	5lb cock, broodstock	2019	4lb	na	na
ICO084_ICY541b	2019	4lb cock, broodstock	08/10/2019	4 lb cock, McNair's	na	na
ICO087_ICO088	2019	5lb cock, unused broodstock	2019	8lb cock,, unused broodstock	na	na
ICY516_ICY543	2019	3lb cock, (2nd clip)	18/10/2019	5lb cock, House Pool	na	na
ICY527_ICY572	2019	3lb Avenue	26/09/2019	3lb grilse, Avenue	na	na
ICY528_ICY545	2019	9lb Generals Pool	18/10/2019	12lb cock, Junction Pool	na	na
ICY530_ICY687	2019	None	03/07/2019	3lb hen, Seat Pool	na	na
ICY717_ICY731	06/10/2018	5lb cock, Junction	24/10/2018	4lb hen, Cruives	na	na
ICY503_ICY704	21/02/2019	6lb hen, kelt Cruives	01/02/2020	6lb hen, kelt Cruives	na	na
ICY541g_ICY614a	10/07/2019	3lb McNairn's	23/10/2019	4lb cock, McNair's	na	na
ICY592_ICY614b	10/07/2019	3lb hen, Corver Pool	08/09/2019	4lb cock, Power lines	na	na
ICY540b_ICY677	15/07/2019	3.5lb hen, MacClean's	28/10/2019	3.5lb hen, Lodge	na	na
ICY581_ICY640	24/07/2019	5lb cock, Rickety Bridge	16/09/2019	5lb cock, above Rickety Bridge	na	na
ICY526_ICY634	01/08/2019	12lb cock, Bridge Pool	23/08/2019	12lb hen, Bridge Pool	na	na
ICY506_ICY533c_ICY684	02/07/2019	5lb hen, Pawson	20/08/2019	5lb hen, Pawson	01/02/2020	3lb hen kelt Cruives
ICY599_ICY615	23/08/2019	10lb cock, Narrows	06/09/2019	10lb cock, above Rickety Bridge	na	na
ICY557_ICY590	11/09/2019	6lb cock, Rock Pool	12/10/2019	7lb Rock Pool	na	na
ICY501_ICY541l_ICY570	28/09/2019	4lb cock, Power Lines	25/10/2019	4lb cock, Chishoun's	01/02/2020	4lb cock kelt Cruives
ICY541e_ICY551	04/10/2019	6lb cock, above Rickety Bridge	14/10/2019	3lb cock, House Pool	na	na
ICY536_ICY541h	24/10/2019	4lb cock, above Rickety Bridge	06/11/2019	4lb cock, House Pool	na	na

Table 3: Number of fish with one or both parents identified within the 2014 broodstock.

			Attadale 2014		Uist 2014		Wild 2014		All Broodstock 2014	
Class	**Possible broodstock years**	**Analysed**	**One parent**	**Both parents**	**One parent**	**Both parents**	**One parent**	**Both parents**	**One parent**	**Both parents**
2016 1yr smolt	2013, 2014	15	1 (6.7%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (6.7%)	**6.7%**	**13.3%**
2017 2+yr smolt	2014, 2015	59	2 (3.4%)	4 (6.8%)	1 (1.7%)	9 (15.3%)	1 (1.7%)	8 (13.6%)	**6.8%**	**35.6%**
2017 adult	2012, 2013, 2014	103	6 (5.8%)	0 (0.0%)	1 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**6.8%**	**0.0%**
2018 adult	2013, 2014, 2015	166	5 (3.0%)	11 (6.6%)	6 (3.6%)	15 (9.0%)	2 (1.2%)	20 (12.0%)	**7.8%**	**27.7%**
2019 adult	2014, 2015, 2016	217	34-38 (15.7-17.5%)	4 (1.85%)	4 (1.8%)	9 (4.1%)	5 (2.3%)	8 (3.7%)	**21.2%**	**9.7%**
Total		**560**	**51 (9.1%)**	**20 (3.6%)**	**12 (2.1%)**	**33 (5.9%)**	**8 (1.4%)**	**37 (6.6%)**		
Retained as brood fish			11	2	2	7	0	4		

Figure 1: reconstructed pedigrees of all fish inferred to have at least one parent from the genotyped 2014 broodstock. Parental fish are shown at the top and connected by lines to their identified offspring shown at the bottom. Offspring in the same full-sibling family are stacked together. Different sexes and fish groups are shown by different shapes and colours as explained in the key. Red squares indicate fish with an estimated aquaculture ancestry > 10%.

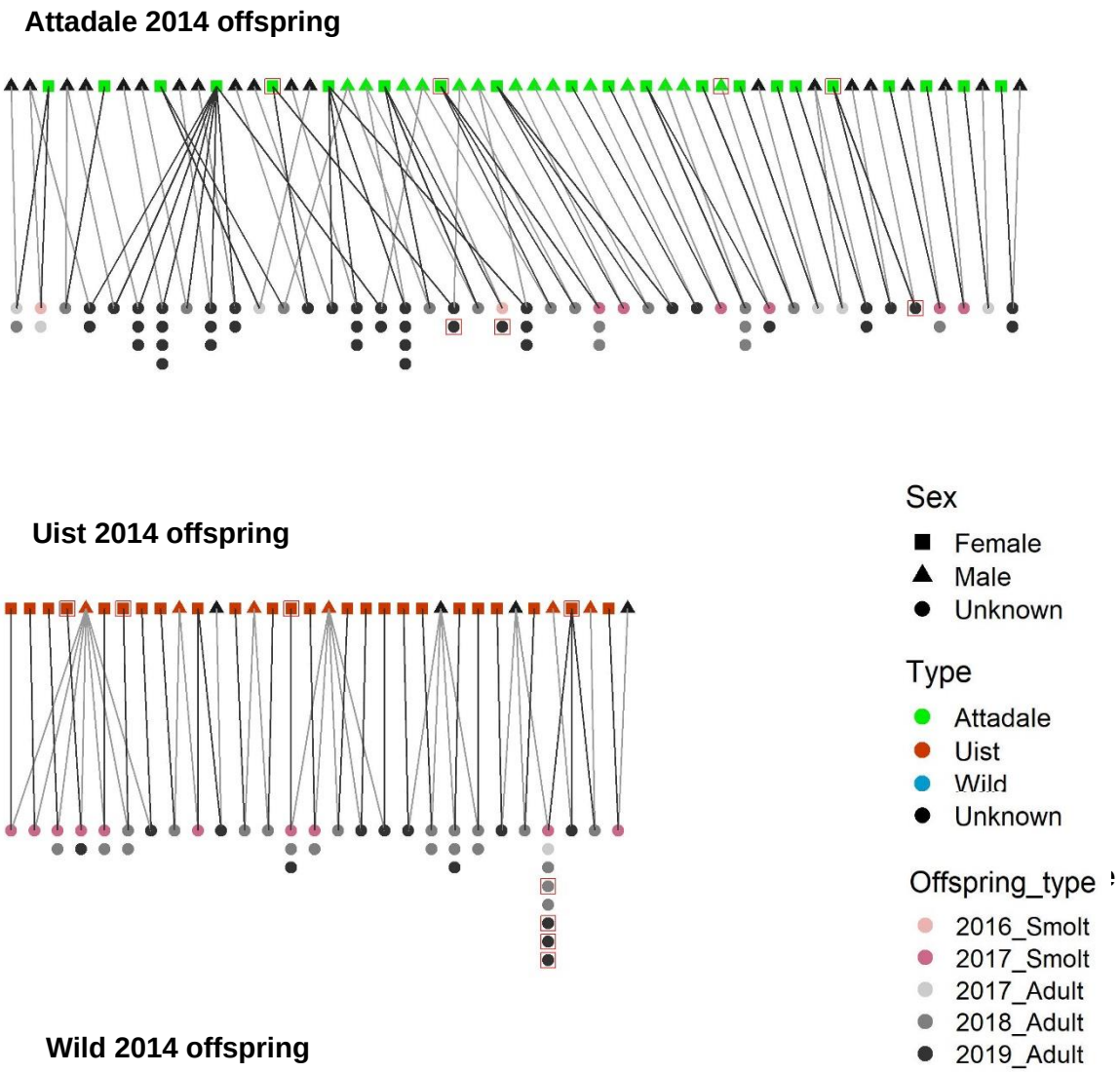

Figure 2: reconstructed sibling relationships among Attadale, Uist and Wild broodstock fish. Red squares indicate fish with an estimated aquaculture ancestry > 10%. Note that the A

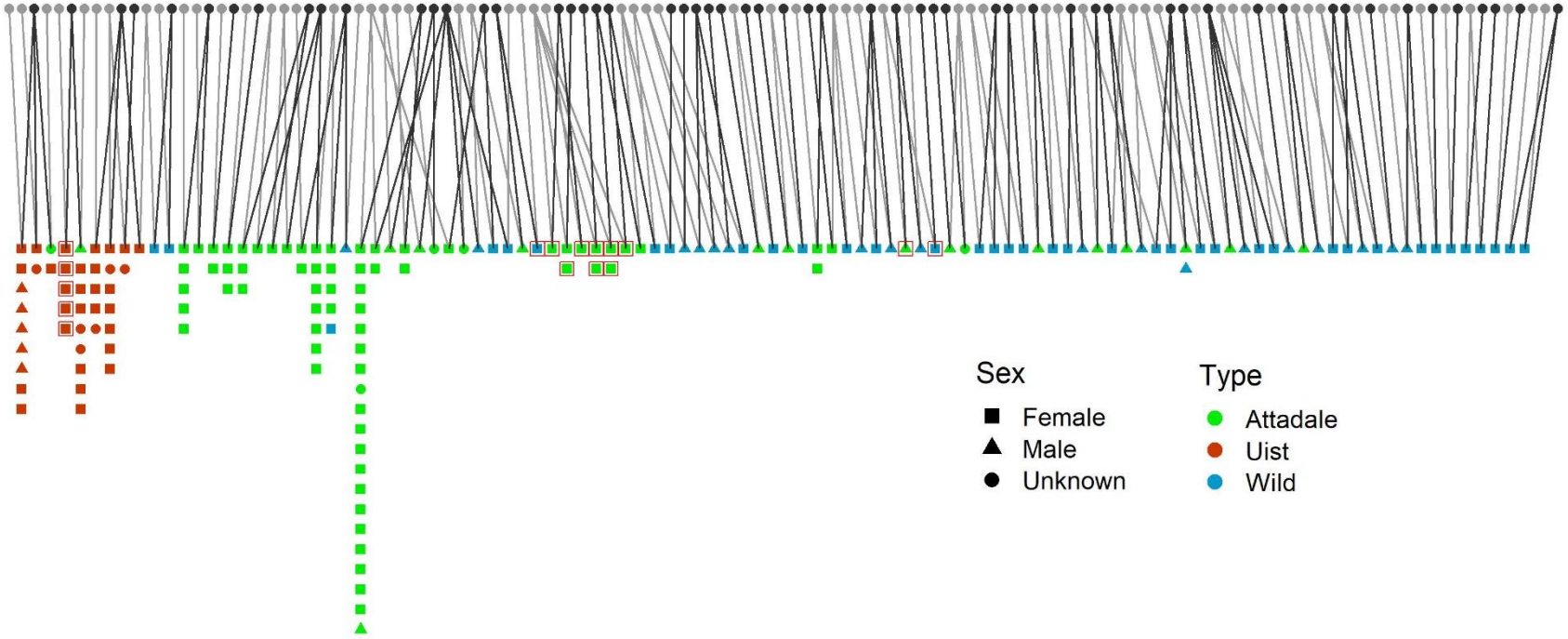

Figure 3: Estimated proportion of ancestry of from Scottish (blue) and Norwegian aquaculture fish (orange). Each column represents an individual fish.