

River Carron Catchment Scale Restoration Programme Phase IIc: Origin of 2016-2018 Carron Smolts

UHI Institute for Biodiversity & Freshwater Conservation, UHI Inverness.

Project Lead: Victoria Pritchard

Project Support: Silvia Ferreira Carvalho, Charley Clark, Matthew Curran, Dasha Svobodova

Project Sample & Data Collection: Robert Kindness (RCCA)

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

Background

Starting in 1994, the River Carron Improvement/ Conservation Association (RCCA) has been undertaking a programme of supplementary stocking with the aim of supporting the Atlantic salmon population in the river. To monitor the impacts of this programme, the RCCA has collected a rich dataset that includes detailed cross and stocking records, results of electrofishing surveys, smolt trapping, adult rod catches and coded wire tagging of stocked juveniles, and thousands of genetic samples taken from broodstock fish, juveniles, smolts and returning adults. This dataset provides the opportunity to understand of how stocking aimed to support and restore a small population of native Scottish salmon has actually impacted that population, and in turn to shape future conservation stocking efforts.

Recently, the UHI Institute for Biodiversity and Freshwater Conservation (IBFC) investigated stocking outcomes by undertaking genetic analyses on broodstock fish and returning adults caught in the river. Detailed information about this study is provided in the previous report '*River Carron Catchment Scale Restoration Programme Phase II: Impact of 2011-2017 Stocking Programme*'. Parentage analysis, linking rod-caught adults to their possible broodstock parents, allowed almost complete discrimination of stocked and wild-spawned adults returning to the Carron in 2017-20. This revealed that 36%-53% of captured returners in each year were stocked as juveniles. However, non-stocked fish contributed proportionately more to the genetic size of the Carron Atlantic salmon population than stocked fish, and one broodstock approach that re-used female parents reduced the overall genetic size of the population in comparison to a non-stocking scenario. We were unable to assess whether stocking reduced the survival of wild-spawned juveniles in the river, or whether stocked and wild-spawned individuals had differential survival at sea or different reproductive success on their return.

This new phase of the study (Phase IIc) starts to explore marine survival by comparing the proportion of stocked individuals in annual samples of out-migrating smolts to the proportions already found for the returning adults. We analyse a set of smolts trapped in 2016-2020 and identify stocked fish by linking them to their 2012-2018 possible broodstock parents. This allows us to address the following questions:

1. What proportion of captured smolts were originally stocked as juveniles, and how does this compared to stocked proportions in returning adults?
2. What is the age of the out-migrating smolts?
3. How many parents produced the wild-spawned smolts and returning adults?

4. What is the level of Norwegian-origin aquaculture ancestry in the stocked and non-stocked smolts?

Methods

Full details of genetic methods and statistical analyses are provided in the previous report.

Genetic Samples: IBFC was provided with tissue samples from smolts out-migrating in 2016, 2017 and 2018 (Table 1). Smolts were caught using a screw trap situated in Brabourne's Pool and fin clips were taken from 20 smolts per day across 10 consecutive days, resulting in a total sample size of 200 smolts per year. Clips were stored in ethanol with pre-allocated unique IDs. Exact capture dates and smolt lengths were also recorded, however as IBFC did not have these data at the time of reporting they have not been incorporated into the analyses.

DNA extraction and genotyping: DNA was extracted from fin clips via alkaline lysis (Truett *et al.* 2000). An established genotyping-by-sequencing approach (Bradbury *et al.* 2018) was then used to genotype each sample for a panel of 88 short tandem repeat genetic markers. MEGASAT (Zhan *et al.* 2017) with custom IBFC input was used to call genotypes. Samples genetically identified as brown trout, trout-salmon hybrids or replicates of another sample were removed from the analysis, as were samples with >25% missing genotypes. Any genetic marker with >20% missing genotypes was also removed.

Identification of stocked fish using parentage analysis: COLONY 2.0.6.6 (Jones & Wang 2010) was used, as previously described, to identify smolts that were the offspring of genotyped broodstock fish. All 2016-18 smolts were tested against all genotyped 2011-2016 broodstock (i.e. the parents of stocked eggs hatching 2012-2017). Any smolt linked to two broodstock parents was classified as stocked. Smolts linked to a single broodstock parent were checked against crossing records, known missing broodstock parents, and returning adult parentage to class them either as a stocked fish with a non-genotyped parent or a wild-spawned individual. We applied the following parameters for all COLONY analyses: 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; no inbreeding; full sibship not scaled; no sibship prior; unknown population allele frequency; combined pairwise likelihood and full likelihood (FLPS) algorithm with medium run length and medium precision.

Identification of Uist offspring using sibship reconstruction: As samples from 2015 and 2016 Uist broodstocks had not been retained, but genotypes of their 2016 and 2017 hatchery offspring were available, additional COLONY analyses were performed to identify smolts that were siblings of these Uist hatchery fish. Smolts that had already been identified as stocked were removed. Two initial rounds of sibship reconstruction were performed, one including the remaining smolts and 161 offspring of Uist 2015 broodstock, and the other with the smolts and 342 offspring of Uist 2016 broodstock. Due to previously described difficulties in genotyping the Uist juveniles, these analyses were based on a reduced number of genetic markers (53). An additional COLONY analysis was therefore performed to reconstruct sibships between smolts and returning adults previously identified as stocked Uist fish, using all markers. Combined results from these three analyses were used to identify smolt offspring of the 2015 and 2016 Uist broodstock parents and to assign them to different Uist juvenile batches, which were released into the Carron at different development stages. Note that for 2016 Uist juvenile batches we are only able to identify individuals smolting at the age of 1-Winter as we have not genotyped smolts past 2018.

Family relationships between non-stocked smolts and adult returners: To further characterize the wild spawning Atlantic salmon population in the Carron, COLONY analyses were also used to explore whether the non-stocked 2016-2018 smolts were the offspring of returning adults caught in the Carron in 2014-2016. We also used COLONY to examine the sibship relationships among the non-stocked smolts and returning adults, limiting this to the years where we could confidently discriminate stocked and non-stocked fish (smolts 2016-2018, adults 2017-2020).

Genetic introgression from aquaculture fish: As before, the presence of genetic material from Norwegian-ancestry aquaculture fish was assessed using the program STRUCTURE (Pritchard *et al.* 2000). We combined the entire post-QC genetic dataset of Carron adults and smolts with a dataset of 600 aquaculture fish from three different aquaculture strains farmed on the Scottish west coasts, genotyped for the same genetic markers. We then used it as input to STRUCTURE specifying two possible ancestral groups (aquaculture vs. wild) with the following parameters: admixture model with uncorrelated allele frequencies, no prior population information, 50,000 burn-in followed by 100,000 MCMC reps; all other parameters default. We examined the overall patterns of estimated aquaculture ancestry across all Carron fish.

Results

Genotyping success: 591 out of 600 smolts were successfully genotyped (Table 1). One of these was identified as a hybrid between salmon and brown trout. Two smolts, from 2016 and 2018, genetically matched returning adults caught in subsequent years (2016 smolt ICE360 = 2018 adult ICY695; 2018 smolt ICM239 = 2020 adult ICO209, Table S1). Four microsatellite markers were removed due to too much missing data, leaving 84 for analysis.

Identification of stocked smolts: Parentage analysis matched 201 out of 590 smolts to a broodstock parent pair (Table 2, Table S1). An additional ten smolts were matched to only one broodstock parent; one was subsequently linked via crossing records to a known non-genotyped broodstock parent and classified as stocked, while the rest were classified as non-stocked, likely with a parent closely related to a broodstock fish. Sibship reconstruction identified a further 91 smolts as the offspring of Uist 2015 or 2016 broodstock, with substantial variation in the recapture rate for different Uist 2016 batches (Table 3). Thus, a total of 237 (40.0%) of 2016-2018 genotyped smolts were originally stocked into the Carron as juveniles (Table 2). Neither of the two smolts re-caught as returning adults had been stocked.

Parentage reconstruction – wild parents: Fifteen of the 353 non-stocked smolts (4.2%) were matched to candidate wild parents that had been caught and released in the river. Most were matched to a single candidate parent, meaning that the confidence of such matches is lower than if both parents had been matched (Table S1).

Age at smoltification and return: Inferred age at smoltification for stocked fish ranged from 1-Winter to 3-Winters (Table S1). As only three years of smolt runs were examined, a complete smolt age profile could only be obtained for the offspring of the 2014 broodstock; 92% of these smolts out-migrated in 2017 at 2W, with 4% each out-migrating in 2016 (1W) and 2018 (3W). Incomplete smolt age profiles for 2013 broodstock offspring (1W smolts not examined) and 2015 broodstock offspring (3W smolts not examined) also suggested that most of these fish out-migrated at 2W. Data from eight years of adult returns (2014-2021) generated in the previous genetic studies showed >90% of stocked fish to return at ages 3W or 4W, with varying proportions of these two age classes across years. Thus, most stocked individuals spent 1-2 years at sea before returning to the Carron to spawn. Age at smoltification and return of wild-spawned Carron fish could not be reliably quantified due to

the low numbers of fish with confirmed wild parents; the very limited data suggests that peak smolt age is also 2W (Table S1)

Inferred number of wild spawners producing sampled smolts: The reconstructed pedigree for non-stocked 2016-2018 smolts (Table S2) inferred that the 353 fish were the offspring of 401 different wild-spawning parents, 176 of which also had offspring among the 2017-2020 returning adults. We observed 36 groups of 2-7 full siblings within the wild-spawned smolt dataset; most but not all of these individuals smolted in the same year as their siblings.

Aquaculture introgression: Figure 1 shows the proportion of aquaculture ancestry estimated for smolts captured 2016-2018 and returning adults captured 2017-2020. As previously observed in the returning adults, putatively wild-spawned and stocked smolts both had a small amount of aquaculture ancestry (mean estimates: stocked smolts 4.7%; stocked adults 4.7%; non-stocked smolts 4.5%; non-stocked adults, excluding three likely direct farm escapes, 4.8%).

Discussion

Here, we have extended our genetic analysis of the extensive Atlantic salmon tissue archive held by the River Carron Conservation Association to compare the stocking origins and aquaculture ancestry of out-migrating smolts to those previously found for returning adults. We focused on the smolt runs of 2016, 2017, and 2018, for which we had genotyped all likely broodstock parents or siblings and could thus reliably discriminate stocked vs. wild spawned fish.

One year's smolt run does not directly correspond to a single year's adult return due to variation in the time that salmon spend at sea before maturing. For this reason, we did not perform a statistical test to compare stocked and wild proportions between life stages. Additionally, as the complete smolt run was not sampled, any difference in smolt timing between stocked and wild-spawned fish (e.g. Kennedy *et al.* 2012) could bias our results. Nevertheless, the majority of the 2016-2018 smolts are expected to return as adults in 2017-2020, and the 10-day sampling design at least partly accounts for inter-individual variation in smolt timing. Examining proportions of stocked vs. wild spawned fish, we find 40.0% stocked fish among the 2016-18 smolts compared to 46.6% stocked fish among the 2017-2020 adults. Thus, for the time period examined, our results do not indicate any reduced marine survival of stocked fish compared to wild-spawned fish.

Several previous studies have compared the marine survival of wild-spawned and stocked Atlantic salmon. Most of these have examined fish reared in captivity until the smolt stage and have found lower return rates for stocked vs. wild spawned fish (e.g. Jonsson *et al.* 2003; Kallio-Nyberg *et al.* 2014). There is rather little data on the comparative marine survival of hatchery-produced fish stocked out at earlier stages, as was done in the Carron. Jokikokko *et al.* (2006) and Kallio-Nyberg *et al.* (2011), correcting for size at smoltification, found little difference in marine survival between wild-spawned salmon and hatchery offspring released into the same Baltic river as parr. In comparison, the size-corrected survival of fish stocked as smolts was much lower. Our own observation of equivalent return rates for stocked and wild-spawned fish is therefore not unexpected. Additionally, the very low numbers of adult returners when the stocking programme was implemented means that most adults returning to Carron are expected to have 'stocked' ancestors and this may be another reason why we do not observe survival differences between the two groups.

For the stocked fish originating from 2014 broodstock crosses, we were able to examine the entire expected range of smolt ages (1-Winter, smolting in 2016, to 3-Winter, smolting in 2018), finding a peak smolt age of 2-Winters. Although we were unable to estimate the age of most of the wild-

spawned smolts, the 2004-2008 River Carron Fisheries Management Plan (Wester Ross Fisheries Trust 2004) references a 1938 study that described a majority 2-Winter smolt age for wild Carron salmon. Thus, there is no current evidence for smolt-age differences between wild-spawned and stocked fish. Scale reading of archived Carron scales could shed more light on the contemporary smolt age distribution.

For juveniles stocked in 2017, which include batches of Uist fry that were experimentally stocked at different stages, we only have information on the migration of 1-Winter smolts. Nevertheless, we observe a striking number of juveniles from Uist Batch 3 smolting at this young age. These fish were stocked as late fry and, while we cannot make any firm conclusions without examining the 2019 and 2020 smolt years, it is possible that their better nutritional status at release promoted early smoltification. This has been seen in other systems (e.g. Kennedy *et al.* 2012). As in the earlier report, we caution that the relatively poor genotyping success for the 2017 and 2018 Uist fry may have resulted in some assignment inaccuracies among Uist fry batches.

As previously seen in the adults, both stocked and wild-spawned Carron smolts contain some non-native genetic material from Norwegian-origin aquaculture fish. There is no substantial difference in proportions between the two life stages and thus no evidence that Carron fish with Norwegian ancestry are at a selective disadvantage at the marine stage.

In conclusion, our results suggest that Atlantic salmon stocked into the Carron as juveniles can complete their marine migration as successfully as wild-spawned fish, leading to a substantial stocked contribution to the returning adult population. However, although we acknowledge the observed correlation between the stocking programme and improved adult returns to the Carron, we caution that successful return of hatchery fish does not necessarily mean that stocking has improved the overall status of a salmon population. For example, studies of two different well-monitored rivers have found a negative correlation between the proportion of stocked vs. wild spawners and population productivity, measured as number of smolt or adult offspring per breeder (Jonsson *et al.* 2019; O'Sullivan *et al.* 2020). This may reflect a general pattern, revealed by genetic parentage studies, of stocked salmon having a lower average reproductive success than wild-spawned salmon when both return to the same river. This effect is greatest when fish are stocked as smolts but has been observed even for Atlantic salmon produced by wild-caught broodstock and stocked as fry or parr (Milot *et al.* 2013; Bouchard *et al.* 2022). It is not due to differences in female fecundity, as stocked females often produce more eggs than their wild counterparts (Jonsson *et al.* 2019; O'Sullivan *et al.* 2020) – instead, researchers have linked it to stocked fish being more likely to return as grilse (Milot *et al.* 2013) and/or being less reproductively competitive (Fleming *et al.* 1997, Jonsson *et al.* 2019, Bouchard *et al.* 2022). Additionally, if the release of captive-bred fish reduces the survival of wild juveniles and thus the number of wild spawners whose offspring survive to adulthood, stocking can reduce the genetic size of the population even while increasing the number of returning adults. While we have no current evidence that such a reduction in productivity or genetic size has occurred in the Carron, these negative effects must be considered a potential risk of any Atlantic salmon stocking programme.

We also emphasize that any apparent positive effects of Atlantic salmon stocking in the Carron may not be replicated in other rivers. Of 17 published scientific analyses of similar 'recovery stocking' programmes - that is, stocking the offspring of local wild-caught broodstock with the aim of restoring depleted populations - only 29% found beneficial effects while 30% found some form of negative effect and the rest found no clear effect (McMillan *et al.* 2023). For example, Bacon *et al.* (2015) and Glover *et al.* (2018) showed that 11 years of egg stocking on the Girnock Burn (River Dee) had no

effect on smolt production, despite poor wild egg deposition and survival, and attributed this to restricted parr habitat. The specific characteristics of the Carron – a short, spate-prone West coast river where juvenile densities appear to be limited by redd washouts and the current, hybrid, salmon population may largely originate from stocking – are different from those in many other U.K. salmon rivers where supportive breeding is being considered. Managers of these rivers should therefore proceed with caution.

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Table 1: Samples and genotyping success

Description	Year genotyped	# Genotyped	Missing data	Trout or hybrid	Duplicate	Analysed
2016 Smolt	2021 - 2025	200	7	0	0	193
2017 Smolt	2021 - 2025	200	0	1	0	199
2018 Smolt	2025	200	2	0	0	198

Table 2: Numbers and percentages of smolts and adults matched to broodstock parents, 2014-21

Category	Expected Broodstock Years	Expected Return Years	No Broodstock Match	Attadale Match	Uist Match	Wild Match
2016 Smolt	2012-2014	2017-2019	113 (58.5%)	37 (19.2%)	0 (0.0%)	43 (22.3%)
2017 Smolt	2013-2015	2018-2020	118 (59.3%)	19 (9.5%)	37 (18.6%)	25 (12.6%)
2018 Smolt	2014-2016	2019-2021	122 (61.6%)	27 (13.6%)	35 (17.7%)	14 (7.1%)
2014 Returners	2008-2011	na	186 (97.4%)	0 (0%)	0 (0%)	5 (2.6%)
2015 Returners	2009-2012	na	51 (96.2%)	0 (0%)	0 (0%)	2 (3.8%)
2016 Returners	2010-2013	na	41 (46.1%)	8 (9.0%)	0 (0%)	40 (44.9%)
2017 Returners	2011-2014	na	67 (64.4%)	16 (15.4%)	0 (0%)	21 (20.2%)
2018 Returners	2012-2015	na	82 (49.1%)	22 (13.2%)	23 (13.8%)	40 (24.0%)
2019 Returners	2013-2016	na	115 (53.2%)	45 (20.8%)	35 (16.2%)	21 (9.7%)
2020 Returners	2014-2017	na	32 (47.1%)	11 (16.2%)	20 (29.4%)	5 (7.4%)
2021 Returners	2015-2017	na	39 (68.4%)	2 (3.5%)	10 (17.5%)	6 (10.5%)
All 2016-18 smolts			353 (59.8%)	83 (14.1%)	72 (12.2%)	82 (13.9%)
All 2017-20 adults			296 (53.3%)	94 (16.9%)	78 (14.1%)	87 (15.7%)

Table 3: Numbers of 1+ smolts inferred to originate from each Uist 2016 fry batch

Uist 2017 Batch	#Stocked (x1000)	Stocking stage	# Identified smolts	Recapture rate (x1000)
Fry Batch 1	90	Eggs	4	0.04
Fry Batch 2	150	Eggs	9	0.06
Fry Batch 3	96	Late fry	28	0.29
Fry Batch 4	219	Early fry	5	0.02
Fry Batch 5	32	Un-fed fry	5	0.16

Figure 1: Estimated proportion of ancestry of from Scottish (blue) and Norwegian aquaculture fish (orange) for Carron smolts and returning adults. Each column represents an individual fish.

