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This report presents the results of marine and freshwater scientific work carried out by Marine Scotland Science.

A National Assessment of the Influence of Farmed Salmon Escapes on the Genetic Integrity of Wild Scottish Atlantic Salmon Populations

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Executive summary

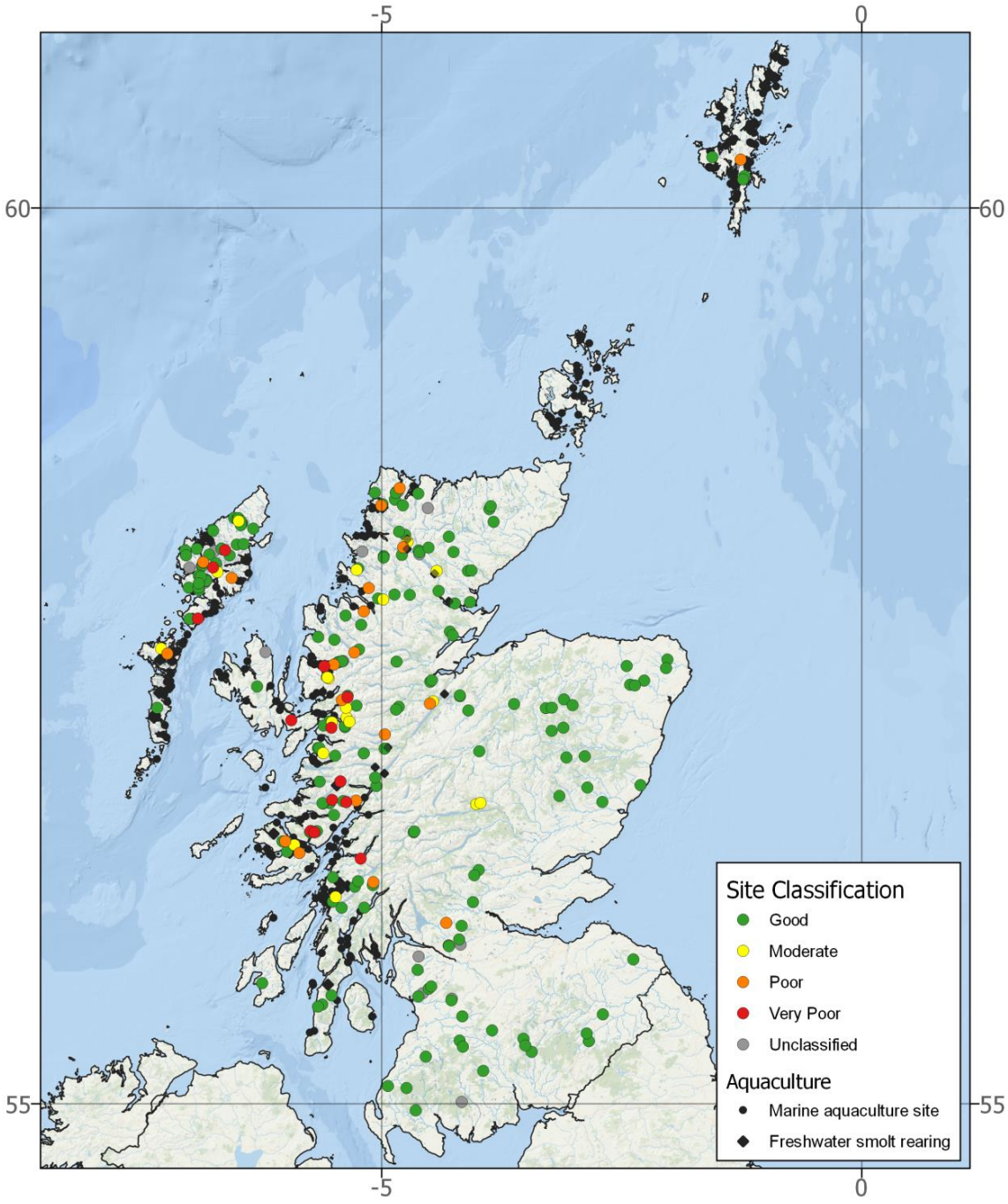
Interbreeding between escaped farmed Atlantic salmon and wild indigenous salmon (hybridisation) introduces genetic material from farmed stocks into wild populations (introgression) with resulting disruption of the adaptive genetic composition of individuals and populations. This can impact their fitness resulting in a significant negative pressure on the viability of wild populations. Recent advances in analytical and statistical techniques are able to differentiate between farmed salmon of Norwegian origin, native wild Scottish salmon and progeny resulting from interbreeding. By sampling a number of juvenile salmon from a particular location it also possible to estimate the proportion of foreign genetic material present in wild Scottish salmon populations and to identify whether this is due to recent or historical events. Monitoring the proportions of wild fish affected by hybridisation is routinely carried out in other countries, and can feed into management decision making. This is the first time a survey to examine the genetic status of populations has been conducted across the geographical extent of Scotland. A bespoke panel of genetic markers, developed specifically to detect genetic changes in Scottish wild salmon, was used to screen tissue samples collected from juvenile fish from 252 sites across Scotland between 2018 and 2019 (n = 2,964 fish). These fish were sampled as part of the National Electrofishing Programme for Scotland (NEPS), and were further supplemented by targeted sampling of sites in the immediate vicinity of freshwater salmon smolt rearing sites. Taken together these data represent the first national scale examination of the genetic integrity of wild juvenile Atlantic salmon in Scotland in relation to interbreeding with Norwegian farm origin salmon strains.

The proportion of wild and farmed (Norwegian) origin genetic material in each sample was used to classify sites using a colour grading system consistent with an approach recently employed in Norway as follows:

- Green – Good condition: No genetic changes observed
- Yellow – Moderate condition: weak genetic changes indicated
- Orange – Poor condition: moderate genetic changes detected
- Red – Very Poor condition: major genetic changes detected
- Unclassified – Fish numbers too low to classify

Of the 252 sites examined, 237 were classified. Of these, signs of introgression were found in salmon at 55 (23.2%) of the sites. Overall classification found 182 (76.8%) sites classified as Good, 21 (8.9%) as Moderate, 20 (8.4%) as Poor, and 14 (5.9%) as Very Poor.

The genetic integrity of populations observed across the country was not uniform. Rather, signs of introgression were concentrated in areas of marine aquaculture production and freshwater smolt rearing. Outside these areas, little to no genetic changes were detected.



Site classification of the genetic status of sampled wild salmon across Scotland in relation to aquaculture production facilities in the marine and freshwater environments. Alternatively coloured version in Appendix 1.

The available evidence indicates that introgression of genetic material from Norwegian farm salmon strains has altered the genetic composition of some populations within rivers near marine aquaculture production. The Shetland Isles, Hebrides and mainland west coast as far south as the Clyde were all notably affected. A substantial number of sites within these regions showed evidence of introgression, however patterns were patchy with nearby sampling sites often unaffected. This fine scale spatial variability in genetic status is similar to that observed in Norway.

With sufficient additional data, spatial regression models have the potential to improve understanding of the various environmental factors affecting the presence and survival of hybrids and could potentially provide a basis for predicting the impacts of changing production locations or intensity on wild populations. Nevertheless, even from these preliminary results it is clear that the presence of marine aquaculture in an area has the potential to affect the overall genetic integrity of local salmon populations.

This study provides further evidence that escapes of juvenile salmon from freshwater smolt rearing facilities can also affect the genetic status of local salmon populations. Three systems were identified as having notable genetic changes in areas with no marine aquaculture. Two of these (Shin and Ness) contain freshwater smolt production, leaving only a single outlier location on the upper Tay that did not have any obvious geographic association with aquaculture.

This report provides an analysis of samples collected as part of NEPS, supplemented by ad-hoc samples (those from near freshwater aquaculture sites). At present all analyses have been conducted only at a site-by-site level. However, the Generalised Random Tessellation Stratified (GRTS) survey design that underpins NEPS will allow these site level results to also be integrated at a regional level in due course. Without this additional work care should be taken in interpreting the precise regional characteristics e.g. proportion of sites affected in different regions. Future work will use the NEPS survey design, in combination with observations of abundance, to quantitatively assess levels of introgression at the regional scale.

Previous studies have shown that farm/wild hybridisation and subsequent introgression carry risks to the health of wild salmon populations. The results presented here should now allow such risks to be better quantified and managed within impacted areas. Further work on the integration of results into a quantitative regional picture, together, importantly, with modelling of the determinants of impacts could increase the understanding of the patterns observed, with the potential for predictive tools to be developed. However, it is important to highlight that these results only represent a simple snapshot of the genetic status of populations.

They do not provide information on any genetic changes caused by on-going farm escapes that can have cumulative effects. Understanding of temporal changes and inter-annual variability will only come from repeated, periodic, structured, surveys.

Effective management of aquaculture and wild salmon stocks under the Scottish Government's Blue Economy sustainable development objectives requires informed science-based decision making. A strong evidence base as to the pressures acting on wild Atlantic salmon is also central to development of the Wild Salmon Strategy. The survey results presented here provide the first national assessment of the genetic integrity of wild salmon populations across Scotland in relation to interbreeding with Norwegian origin strains. This analysis can aid managers and policymakers when making decisions on the sustainable development of the salmon aquaculture whilst also conserving wild salmon populations.

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Background

Interbreeding between escaped farmed salmon and wild conspecifics, and the resulting introgression of genetic material from farm stocks into the wild, brings risks to the diversity, genetic integrity, fitness and viability of wild salmon populations (Naylor et al., 2005; Glover et al., 2020). Selective breeding programs, targeting important commercial traits (Thodesen et al., 1999; Gjedrem and Baranski, 2009; Solberg et al., 2013), together with unintentional hatchery domestication (Hutchings and Fraser, 2008; Karlsson et al., 2011), have resulted in aquaculture strains that are genetically and phenotypically very different from wild stocks (Christie et al., 2016; López et al., 2019). These genetic changes make such fish substantially less fit when released into the wild compared to their wild conspecifics (Fleming et al., 2000; McGinnity et al., 2003; Skaala et al., 2012; Besnier et al., 2015; Reed et al., 2015; Skaala et al., 2019). In addition, around 90% of aquaculture salmon production in Scotland uses strains of Norwegian origin (Munro, 2020) which are genetically distinct from Scottish stocks (Gilbey et al., 2018b) and interbreeding of fish from such divergent, genetic groups results in outbreeding depression and associated reduced fitness (Côte et al., 2014 and references therein). Interbreeding of escaped farmed fish of Norwegian origin with wild Scottish fish thus has the potential to negatively impact wild populations. Such effects can persist across subsequent generations and can be increasingly detrimental if the interbreeding is cumulative and frequent (McGinnity et al., 2003; Glover et al., 2017; Castellani et al., 2018).

Genetic changes in native salmon populations, as a result of interbreeding with farmed escapes, have been observed in Ireland (Crozier, 1993; Clifford et al., 1998b; Clifford et al., 1998a; Crozier, 2000), North America (Bourret et al., 2011; Wringe et al., 2018; Sylvester et al., 2019) and Norway (Glover et al., 2012; Karlsson et al., 2016; Glover et al., 2017). In Scotland evidence of farm/wild hybridisation was previously observed in a limited study focused on the west coast where 25% of the fish examined were classified as farm/wild hybrids (Coulson, 2013).

Monitoring for evidence of hybridisation between wild and farmed salmon has been aided by the development of a standardised method for quantifying unidirectional gene flow from farm to wild fish (Karlsson et al., 2014). This approach is now an integral part of the management strategy in Norway, where a periodic survey is undertaken to characterise the genetic influence of escaped farmed salmon on wild salmon stocks (Diserud et al., 2017; Diserud et al., 2020). The most recent report (2020) covers 239 wild populations and classifies them using a colour grading system according to the degree of introgression in each population (Diserud et al., 2020). Characterisation of populations in this way allows managers to obtain

a better understanding of farm-wild salmon interactions and has obvious potential for examining the genetic status of stocks in Scottish rivers.

This report seeks to provide, for the first time, an overview of the genetic integrity of wild juvenile salmon populations in Scotland with respect to the proportion of genetic material in each sampled population derived from non-native farmed fish of Norwegian origin. The work uses a bespoke genetic marker panel, developed to provide maximum power to discriminate among native, farmed and hybrid fish, to examine tissue samples collected from across the country. The objective of this report is to provide a site-by-site assessment of the genetic status of salmon populations across Scotland and to assess any spatial relationships with the location of aquaculture production facilities. Information gained from this analysis can be used to inform management decisions associated with the sustainable development of the aquaculture sector and conservation of wild salmon populations under the Scottish Government's Blue Economy agenda.

Materials and methods

Genetic samples

Sites were screened to achieve three objectives: 1) geographic coverage of the whole country; 2) more detailed coverage of the Scottish west coast and Hebridean Islands, the main areas of marine aquaculture production; and 3) targeted coverage of areas around freshwater smolt rearing facilities.

Samples of salmon tissue were collected as part of the National Electrofishing Programme for Scotland (NEPS), which uses a stratified, unequal probability, generalised random tessellation stratified (GRTS) sample design for surveying over time with an oversample (Malcolm et al., 2019; Malcolm et al., 2020). NEPS samples were collected by local fisheries managers or their agents in 2018 and 2019 and each consisted of a small piece (~2x2mm^2) of caudal fin tissue taken under anaesthetic and stored in individually-labelled ethanol-filled tubes. Samples were obtained from 30 salmon (or all fish if less than 30) from each site where quantitative 3-pass electrofishing had been undertaken as part of the NEPS sampling. Full details of standard operating procedures can be found on NEPS webpages:

https://www.gov.scot/publications/national-electrofishing-programme-for-scotland/pages/standard-operating-procedures

The genetic screening used a sub-set of the available NEPS sites and/or samples. Firstly, to give an unbiased picture across the whole country, 3 sites were chosen at random from

each of the 27 NEPS regions (Malcolm et al., 2019; Millidine et al., 2019) where tissue samples were available. From each of these sites a random sub-sample of 15 fish were chosen at random to give 45 fish per region. If there were less than 15 samples at a site, additional sites were added at random to make up the required 45 fish per region. Secondly, to provide more detailed spatial representation of the Scottish west coast and Hebridean Islands, all available sites were screened from the regions containing marine aquaculture activity. Thirdly, targeted “ad-hoc” sites were sampled in rivers near to freshwater smolt rearing sites during 2018. These sites were chosen to represent rivers in the vicinity of active freshwater smolt rearing locations based on information provided by the Fish Health Inspectorate at the Marine Scotland, Marine Laboratory, Aberdeen. Exact sample site locations were established after liaison with the local River and Fishery Trusts to maximise the chances of rapidly obtaining target sample numbers but did not conform to any formal survey design.

A final set of samples was provided by the Scottish Environmental Protection Agency, collected as part of their summer electrofishing programme and covering areas with no or low NEPS coverage (including Shetland). This represented a second source of ad-hoc samples. All genetically screened sample sites are shown on Figure 1.

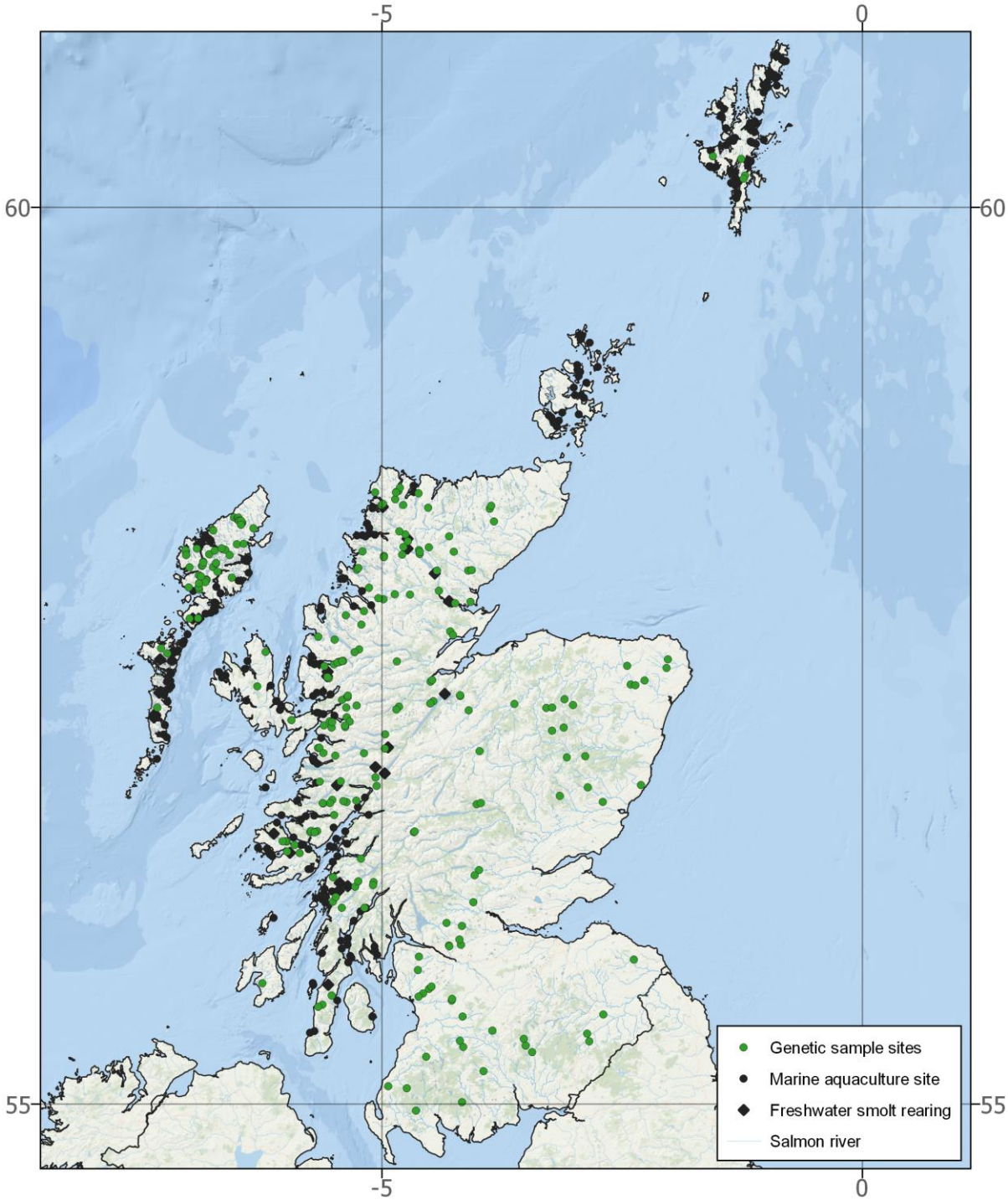

Figure 1. Sample sites where genetic screening was carried out in and marine and freshwater aquaculture production facilities.

Genotyping

DNA was extracted from fin tissue using a Chelex extraction protocol (Walsh et al., 1991). Fish were screened using the bespoke panel of 74 Single Nucleotide Polymorphic markers (SNPs) developed to discriminate between native salmon, farmed salmon of Norwegian origin and their hybrids (Gilbey et al., Submitted). Genotyping was carried out on a Fluidigm

EP1 platform (Fluidigm, San Francisco, CA, USA) following the manufacturer's protocols. Screening consisted of 3031 fish from 252 sites with a mean number of fish per site of 11.7 ± 7.2. Genotyping success of individual fish was examined and those with low (>33% loci not scored) or no scoring success were removed from further analysis. Mean genotyping success of the remaining fish was 98.7% ± 2.8.

Family structure

The family structure of the samples was examined at each site. This is an important step as samples containing a large number of full-sibs (sharing both parents) would require a different interpretation from samples where all of the fish were derived from different families. For each site, the presence of full-sibs was examined using maximum likelihood estimations as implemented in the COLONY software package (Jones and Wang, 2010).

No Full-sib families	No of sites	Mean family size	Max family size
0	178	-	-
1	46	2.2	4
2	19	2.5	5
3	3	2.0	2
4	4	2.3	4
5	2	2.0	2

Table 1. Full-sib family structure across all sites. No Full-sib families refers to the number of families of at least two full-sibs at a site. No of sites are the numbers of sites with the defined number of Full-sib families. Mean family size is the average full-sib family size at sites with at least 1 full-sib family present. Max family size is the largest number of full-sibs at a site.

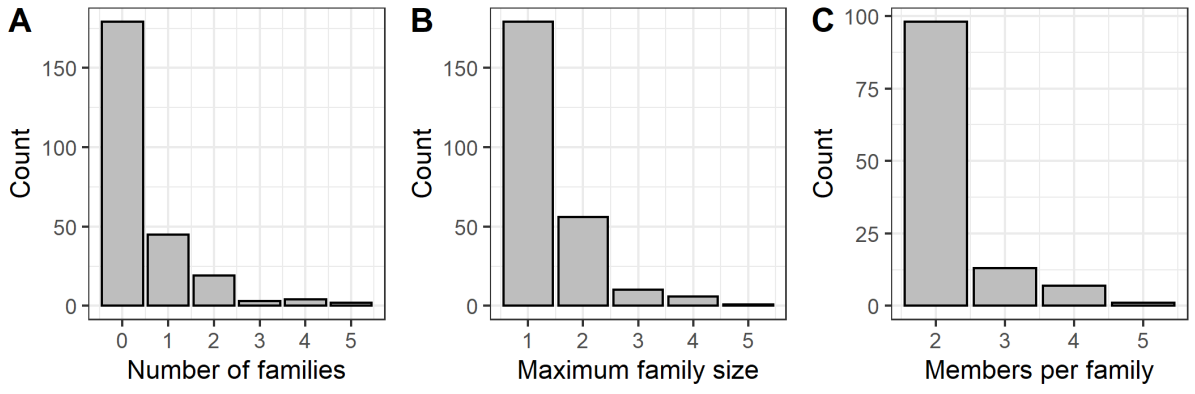

Figure 2. Summary of the number of full-sib families and the number of members of each family at each site. A) Number of full-sib families per site; B) Maximum family size per site (largest full-sib group at a site); C) Number of members per family over all sites (number of individuals in a full sib family). Count is the number of sites in each category.

Analysis of family structure in the samples revealed that at most sites (70.6%) there were no full-sib families (Table 1, Figure 2) i.e. all fish had at least one unique parent. Where there were full-sib families present, in most cases, these consisted of a single sib-pair (1 family; 18.3%) or two such pairs (2 families; 7.5%), with an average family size slightly above the single pair level (Table 1). As such, any general pattern regarding genetic integrity seen across the country would not be expected to be significantly influenced by the inclusion of large single families. At a site-level, however, care must still be taken in the interpretation of the results, especially where the sample numbers were low. Full details of the family structure at each sampled site are contained in the relevant regional breakdowns below and in Appendix 2.

Genetic status

Calculation of P(wild)

The proportion of the genome of an individual fish that is of Norwegian farm strain origin can be estimated by comparing the genetic signature of that fish against two sets of reference samples representing farmed and wild fish. From this genetic assignment of reference individuals, it is possible to assign each individual a probability of belonging to the wild *P*(wild) reference sample. The corresponding probability of belonging to the farm centre point is 1 – *P*(wild). Probability distributions range from around 1 for pure wild fish to distributions around 0 for pure farmed fish. Farm/wild hybrids will fall somewhere between these two values depending on the proportion of its genome originating from the two parental types (Karlsson et al., 2014; Gilbey et al., Submitted).

The genetic status of individual fish was determined by assigning each fish to farm and wild reference samples following the procedure described by Karlsson et al. (2014). In brief, each fish was compared to two panels of 100 farm and 100 wild *in silico* generated reference centre points (computer generated matings of random pairs of individuals from the reference populations) created from farm and wild reference fish (Gilbey et al., Submitted) where the reference centre points are in Hardy-Weinberg equilibrium which is important for unbiased genetic assignment (Kalinowski, 2011; Karlsson et al., 2014). Subsequently, the probability of belonging to the wild centre point *P*(wild) was determined for each fish.

P(wild) was calculated using a systematic Bayesian clustering approach applying Markov Chain Monte Carlo (MCMC) estimation as implemented in the STRUCTURE software package (Pritchard et al., 2000). This was performed using 50,000 repetitions as burn-in, followed by a further 100,000 repetitions with no *a priori* information of sampling locality/origin, and assumed two populations (wild and farm). Each fish was analysed

separately, together with the two farm and wild reference populations, to prevent biases that may be introduced if all samples were included in a single analysis (Kalinowski, 2011; Karlsson et al., 2014). For each fish, the probability of belonging to the wild centre point *P*(wild) was individually calculated and recorded.

Identifying wild and hybrid fish

To define a cut-off for the identification of individual hybrid fish, estimated *P*(wild) for the original reference wild and farm populations (Gilbey et al., Submitted) and *in silico* generated F1 and backcross hybrids were examined. These consisted of first-generation crosses (F1) together with backcrosses to the two parental types, farm (BC1F) and wild (BC1W) (Figure 3). Although the choice of SNPs aimed to minimise the impact of phylogenetic variation in the wild reference centre, there was still some regional variation in SNP characteristics. This likely represents a combination of residual wild inter- and intra-population genetic variation, together with the inadvertent incorporation of fish with evidence of some introgressive hybridisation in the wild reference fish utilised (see below for discussion of this topic). This variation is evident in the tail of the *P*(wild) distribution in the wild reference fish (Figure 3).

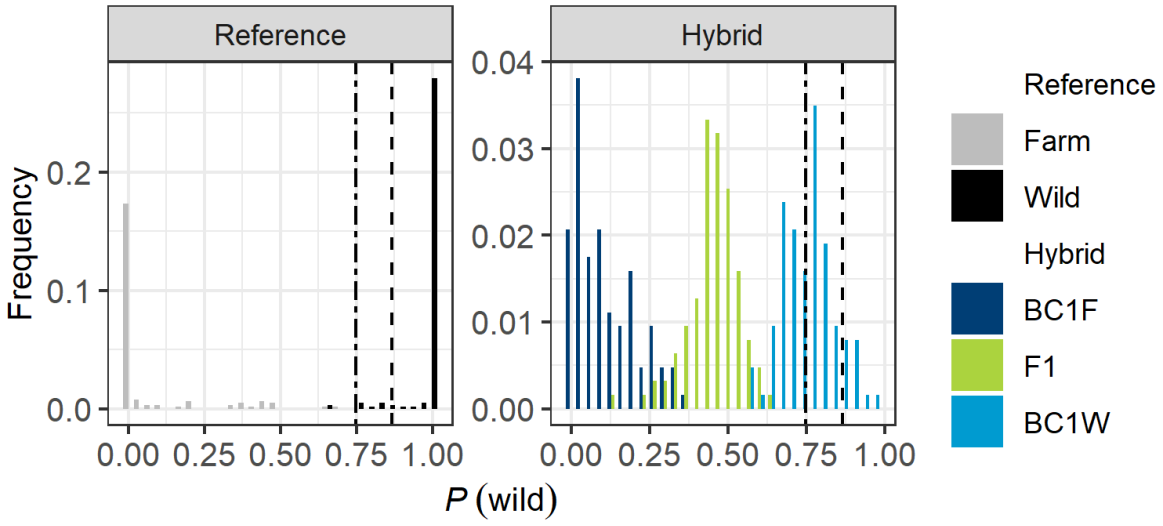

Figure 3. *P*(wild) values for left: original farm (light grey) and wild (black) reference fish; and right: *in silico* generated hybrid fish (BC1F: dark blue; F1: green; BC1W: light blue). Vertical lines represent the lower 1 (long/short dashes) and 5 (regular dashes) wild reference *P*(wild) percentiles. See text for cross definitions.

In this analysis, it is important to avoid categorising wild fish as farmed fish. As such, a strict wild reference 1-percentile *P*(wild) cut-off of 0.747 was utilised here to identify fish sampled in the wild that show evidence of hybridisation. By definition this will mean that, based on the

reference fish, 99% of wild fish will be correctly identified as such. Examination of the *in silico* generated hybrid reference fish (Figure 3) revealed that all farm and all F1 (first generation hybrid) fish would also be correctly identified. However, using this strict cut-off will also likely result in some hybrid fish being classified as wild fish, and indeed a subset (54%) of *in silico* generated BC1W backcross to wild fish (hybrid v wild cross) were not identified using this strict cut-off (Figure 3). When defining a cut-off of this nature there will always be some trade-offs in terms of the accuracy of assignment. In the present analysis, a conservative approach has been adopted to minimise the chance that wild fish are incorrectly classified as hybrids. The results of the analysis should thus be viewed as a minimum estimate of the levels of introgression in the sample of fish obtained across the country.

Estimation of site-level genetic status

To provide a comparison with previously published surveys on the genetic influence of escaped farmed salmon on wild stocks (Diserud et al., 2017; Diserud et al., 2020) this study followed the same statistical approaches. However, for most of the sites examined in this study sample sizes were low, so care must be taken in the interpretation of results. Sample sizes are detailed below in the regional breakdowns and in full in Appendix 2. Because most of the sampling reported here used the NEPS GRTS design, future analysis will aggregate across sites to provide a quantitative regional analysis, thereby reducing the effect of small sample sizes at individual sites.

When examining the levels of farm introgression in a wild population, the null hypothesis is that the wild population has the same mean *P*(wild) as the reference wild populations (i.e. there has been no introgression of genetic material from the farm to the wild) (Karlsson et al., 2014; Diserud et al., 2017). Comparison between the wild reference population *P*(wild) and each population under investigation were performed using one way T-tests on logit-transformed (Warton and Hui, 2011) *P*(wild) probabilities.

Heavy tail

In some situations, there may be a relatively large number of fish of wild origin at a sample site, but a small number showing signs of hybridisation. In such a situation, a simple comparison of means might not be informative. To address this situation, a T-test was performed which compared the *P*(wild) values for the lower 5-percentile of sampled fish to the reference wild *P*(wild) lower 5-percentile. If the 5-percentile was significantly lower than might be expected based on the wild reference samples then this would indicate a set of fish more genetically similar to farmed salmon, and hence provide evidence of hybridisation due

to the lower “heavy tail” analysis (Diserud et al., 2017). For consistency with previous studies, this analysis has again been performed here. However, small sample sizes again require careful consideration when interpreting results.

Levels of introgression

Individual *P*(wild) probabilities at each site were combined to estimate site-level measures of introgression. In a situation where there is a historical reference sample from a population from before aquaculture inputs (as is the case with some Norwegian populations), the two samples (historical and contemporary) can be compared directly by the T-test on two means as described above (Karlsson et al., 2014). However, when evaluating the extent of genetic changes caused by introgression of farm origin genetic material into a wild population without a historical reference sample, as is the case here, the test will have an additional variance component caused by the variability in mean *P*(wild) between wild populations. The variance in wild population mean *P*(wild) values can be estimated from the available wild references which are considered here to be a random sample from all wild populations (Karlsson et al., 2014; Gilbey et al., Submitted). Calibration of the estimate of introgression is required because wild reference populations have an average *P*(wild) less than one, and farm reference populations have an average *P*(wild) above zero. Hence, the proportion of wild genome left in an admixed population, was calculated as (Karlsson et al., 2014):

$$\text{Proportion wild genome left} = \frac{\left(\overline{P(\text{wild})} - \text{Farm}_{\text{ref}}\right)}{\left(\text{Wild}_{\text{ref}} - \text{Farm}_{\text{ref}}\right)}$$

where $\overline{P(\text{wild})}$ is the average *P*(wild) for the population under investigation, Farm_{ref} is the average *P*(wild) for the farm reference samples and Wild_{ref} is the average *P*(wild) for the wild reference samples.

Site classification

Sites were classified based on a combination of criteria derived from the various tests previously described, and following the procedure carried out in Norway for juvenile fish (with some modifications) as outlined by Diserud et al. (2020). The criteria used to classify sites are described below and are the result of an integration of the tests to quantify levels of introgression at a site, together with the number of hybrid fish detected at a site. In contrast to previous studies which imposed a minimum sample size of 20 fish at each site (Diserud et al., 2020), the current study usually had fewer than 20 samples per site. Nevertheless, analysis and classification were undertaken for all sites to provide a preliminary overview of extent and potential levels of introgression across Scotland. In the reporting the results at a site-level, full details of the metrics are reported for each site, together with sample sizes

(Appendix 2). In this way the classifications can be assessed alongside the sample sizes so uncertainty can be evaluated directly.

Classification criteria

Green (condition Good): No genetic changes observed.

Green represents the situation where none of the measures indicate any genetic change has occurred, and no individual fish is classified as hybrid.

Yellow (condition Moderate): Weak genetic changes indicated.

- The probability (P-value) for the one-way t-tests comparing wild reference fish to the fish at the site lies in range <0.05.
- The probability (P-value) for comparing the 5-percentile (tail of the distribution) suggests a significant difference from wild reference fish <0.05.
- There are >0% to 10% of individual fish classified as hybrids at the 1-percentile cut-off.
- If any of the above criteria are met and the estimate of site level introgression is in the range >0 to <6.5%.

Orange (condition Poor): Moderate genetic changes have been detected.

- The probability (P-value) for the one-way t-tests comparing wild reference fish to the fish at the site lies in range <0.05.
- The probability (P-value) for comparison the 5-percentile (tail of the distribution) suggests a significant difference from wild reference fish <0.05.
- There are >10% to 20% of individual fish classified as hybrids at the 1-percentile cut-off.
- If any of the above criteria are met and the estimate of site level introgression is in the range 6.5% to <12.5%.

Red (condition Very Poor): Major genetic changes have been detected.

- The probability (P-value) for the one-way t-tests comparing wild reference fish to the fish at the site lies in range <0.05.
- The probability (P-value) for comparison the 5-percentile (tail of the distribution) suggests a significant difference from wild reference fish <0.05.
- There are >20% of individual fish classified as hybrids at the 1-percentile cut-off.

- If any of the above criteria are met and the estimate of site level introgression is 12.5% or more.

Unclassified

- Sites with a single fish sample

Classification comparisons between Norway and Scotland

The site level of introgression classification used here have been systematised using the same scales as that used in Norway (Diserud et al., 2017; Diserud et al., 2020). However, there are differences between the Scottish and Norwegian situations which must be taken into consideration when making comparisons between the countries.

Firstly, the current study did not require a minimum samples size of 20 fish to undertake a classification. Instead, introgression levels are reported here for all sites with >1 fish. The current study used available material and is intended to provide a preliminary assessment of the genetic integrity of salmon populations within Scotland. As such all screened material was used in classifications. Thus, there should be caution in interpreting results from sites with low numbers of fish. Results from such sites will inevitably be less certain and so should be considered as such. Ongoing analyses will combine sites using the underlying NEPS GRTS statistical survey design to allow estimation of levels of introgression at larger regional scales.

Secondly, the survey classification utilised in Norway is based around estimating levels of introgression mainly using fish at the adult life-history stage. The Scottish survey reported here is based on juveniles. There is evidence that throughout the salmon life-cycle, farm/wild hybrids have differential life-stage survival compared to pure wild fish (Fleming et al., 1996; McGinnity et al., 2003; McGinnity et al., 2004; Skaala et al., 2012; Reed et al., 2015; Castellani et al., 2018; Wringe et al., 2018; Skaala et al., 2019; Sylvester et al., 2019). Such influences must be taken into account when making comparisons between different life history stages such as juvenile to adult, or even different age classes within a life stage (e.g. different aged juveniles). In their recent survey of levels of introgression in Norway, Diserud et al. (2020) used both adults and juveniles to estimate genetic status. To standardise results across life stages, the critical boundary for introgression was raised by 2.5 % for juvenile assessments relative to adults. This was intended to reflect the lower survival of hybrid fish between juvenile and adult life stages, compared to wild fish. The figure was based on an analysis by Karlsson et al. (2016) which compared introgression levels in juvenile and adult fish from the same cohort, in the same river and found an average 2.5%

reduction in levels of introgression between life stages. In the absence of better data, the same correction factors have been applied in Norway and again here. In the future, new data collected in both Norway and Scotland should allow for improved corrections reflecting local variability in demographic structure and other controlling variables (e.g. Wacker et al., 2021).

Levels of introgression

Interpretation of results

The results presented here indicate site-by-site levels of introgression drawn from ad-hoc and formal (NEPS) survey designs. In the case of the former, the choice of sites was deliberately weighted towards locations near to freshwater aquaculture facilities, which are of specific interest. In the case of NEPS sites, the sample design ensured that the sampling is spatially balanced (approximately equally distributed over the area of interest), while maintaining random site selection at finer spatial scales. The following results provide counts and associated percentages of the sites in the different categories reported for the country as a whole and for the various regions within it. The intention of this data summary is not to provide a quantitative unbiased estimate of levels of regional / national introgression, but instead to provide an early qualitative indication of spatial patterns on a site-by-site basis, which will be the first of its kind in Scotland. In future, the NEPS GRTS survey design and associated estimates of abundance will allow a quantitative estimate of levels of introgression (with associated uncertainty) within regionally defined salmon populations. Work is underway in Marine Scotland to complete this more formal analysis.

Country-wide estimate of genetic status

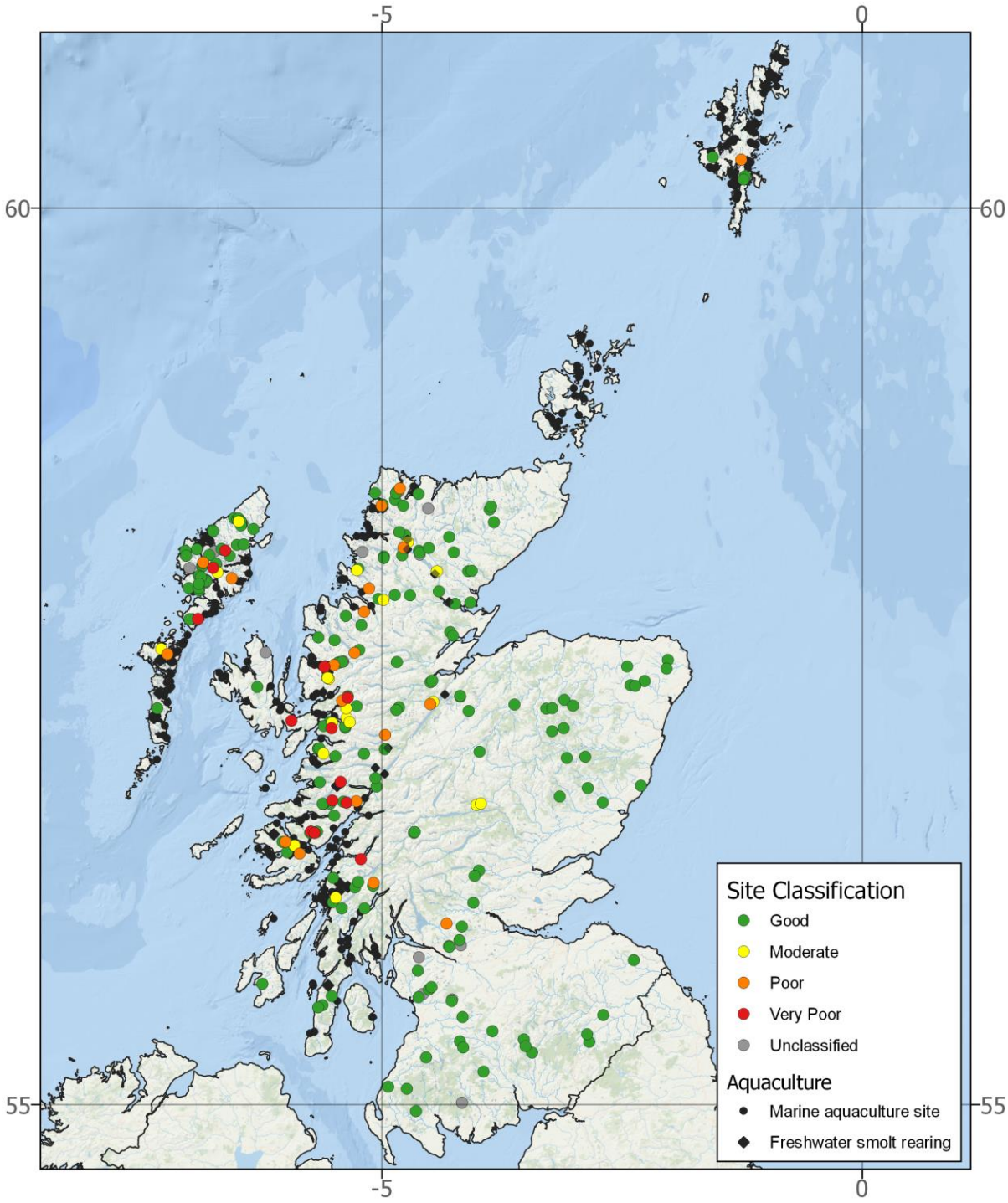

Figure 4. Site classification of the genetic status of sampled wild salmon across Scotland in relation to aquaculture production facilities in the marine and freshwater environments. Alternatively coloured version in Appendix 1.

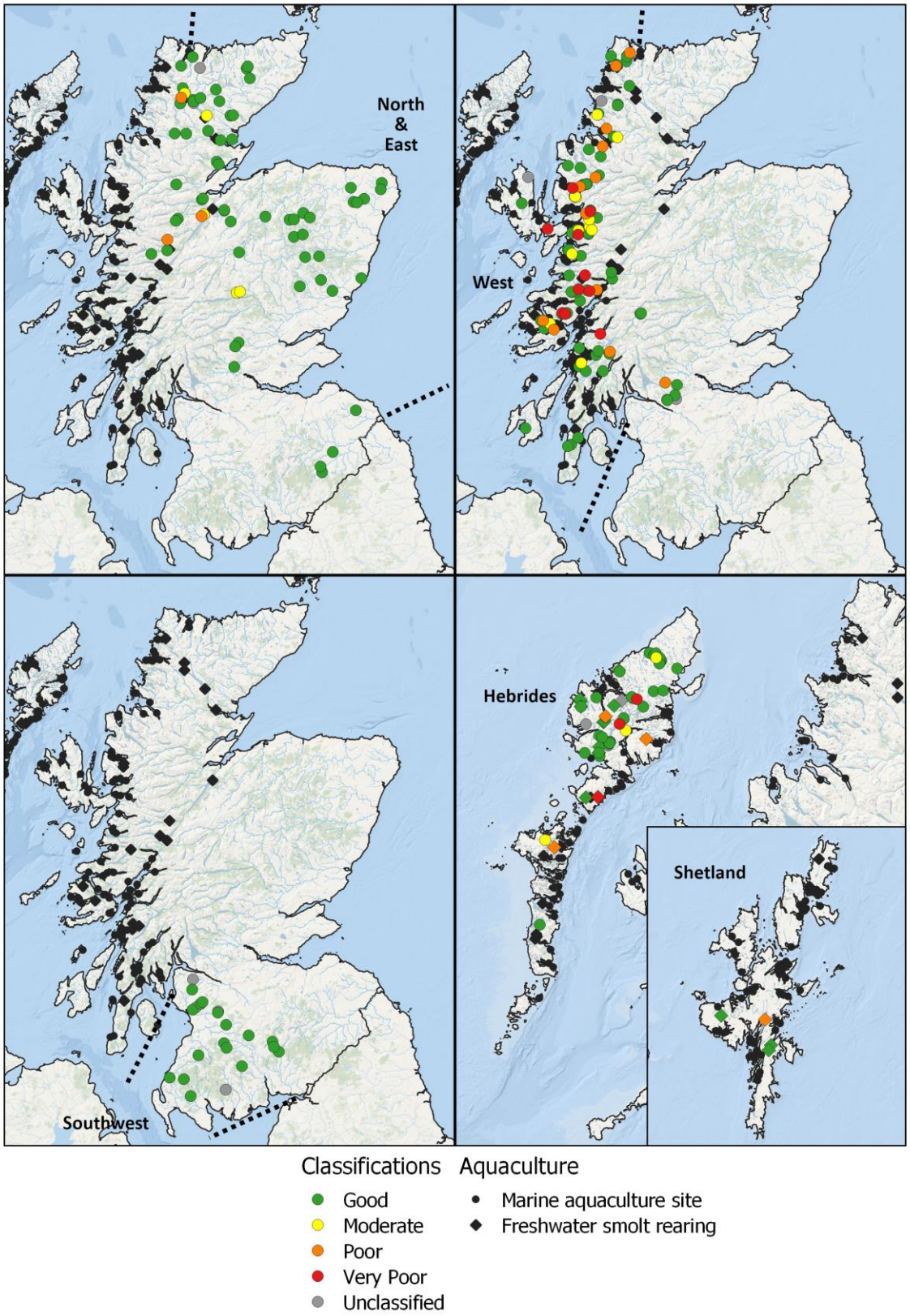

Figure 5. Regional site classification for the genetic status of sampled wild salmon across Scotland in relation to aquaculture production facilities. Where top left is the North and East region; top right is the West region; bottom left is the Southwest and bottom right is the Hebrides and Shetland regions.

Classification	All		North & East		West		Southwest		Hebrides		Shetland	
	n	%	n	%	n	%	n	%	n	%	n	%
Classified	237	94	67	97.1	98	95.1	17	81	51	92.7	4	100
Unclassified	15	6	2	2.9	5	4.9	4	19	4	7.3	0	0
Level	n	%	n	%	n	%	n	%	n	%	n	%
Good	182	76.8	59	88.1	61	62.2	17	100	42	82.4	3	75
Moderate	21	8.9	5	7.5	13	13.3	0	0	3	5.9	0	0
Poor	20	8.4	3	4.5	13	13.3	0	0	3	5.9	1	25
Very Poor	14	5.9	0	0	11	11.2	0	0	3	5.9	0	0

Table 2. Summary of genetic status of Scottish sample sites across the whole country (All) and the various regional splits as shown in Figure 5. Values are numbers (n) and percentages (%) of sites.

Across the country (Figure 4) there is strong evidence for introgression of genetic material originating from Norwegian farm stocks into wild salmon populations. Of the 237 sites classified, 76.8% showed no evidence of introgression, whereas 23.2% had at least some introgression detected (Table 2). Within this overall picture, there was clear regional variability that related directly to the presence of aquaculture facilities (Figure 5) and this has been used to derive the regional summaries reported below.

Regional variability in introgression

There was very little evidence of introgression in wild salmon across the North and East of the country (Figure 5). Within this large area, 88.1% of sites were classified as Good (Table 2). The only two rivers within this region with significant levels of introgression were the Shin and the Ness. Both of these systems contain loch-based freshwater smolt rearing sites. Escaped juvenile farm fish have previously been directly observed in the Shin system (Gilbey et al., 2018a). The only other location within the region where genetic changes were identified was the Errochty Water, an upper tributary of the river Tay where two adjacent sites were categorised as moderate. Closer examination of these data revealed that the classifications were due to the presence of a single fish with a *P*(wild) value just below the 99-percentile threshold (0.739) at one site, and a significant one-way T-test result ($p = 0.020$) for the other site, although no individual fish were classified as hybrids there. The potential reasons for these classifications in the Errochty are further explored below in the detailed regional analysis. With the exception of the Errochty results, the overall picture for the North and East of Scotland suggests no substantial evidence of genetic changes outside of areas associated with freshwater smolt rearing sites.

The West region, to the north of the Clyde had the greatest concentration of sites with evidence of introgression (Figure 5). This area also contains most of the mainland marine aquaculture production in Scotland. Of the 98 sites classified in this region, 37.8% showed evidence of introgression, with 24.5% classified as either Poor or Very Poor (Table 2).

There was no evidence of introgression in the Southwest region (Figure 5). All 17 sites were classified as Good (Table 2). This region lies to the south of the main areas of aquaculture production and contains no marine or freshwater rearing facilities.

In common with the West coast region, a number of sites in the Hebrides, an area with high aquaculture production, had evidence of introgression (Figure 5). Of the 51 classified site samples, 17.6% showed evidence of introgression with 11.8% classified as Poor or Very Poor (Table 2). In Shetland too, evidence of introgression was observed and this region also has substantial aquaculture operations. Of the 4 sites classified, 1 was found to be in the Poor Category.

Detailed regional analysis

The following sections describe, in detail, site-wise patterns of introgression within the NEPS regional sample strata. The NEPS regions within the North and East where very few sites showed any evidence of genetic changes have been combined, as have those in the Southwest.

North and East (excluding freshwater smolt rearing areas)

The large North and East combined region consisted of combined NEPS regions: Brora/Helmsdale, Conon, Dee, Deveron, Don, Esk, Forth, Nairn/Findhorn/Lossie, Northern, Spey, Tay, Tweed, and Ugie (Figure 6). Areas with freshwater smolt rearing sites (Kyle of Sutherland, Ness & Beauly) are examined individually below.

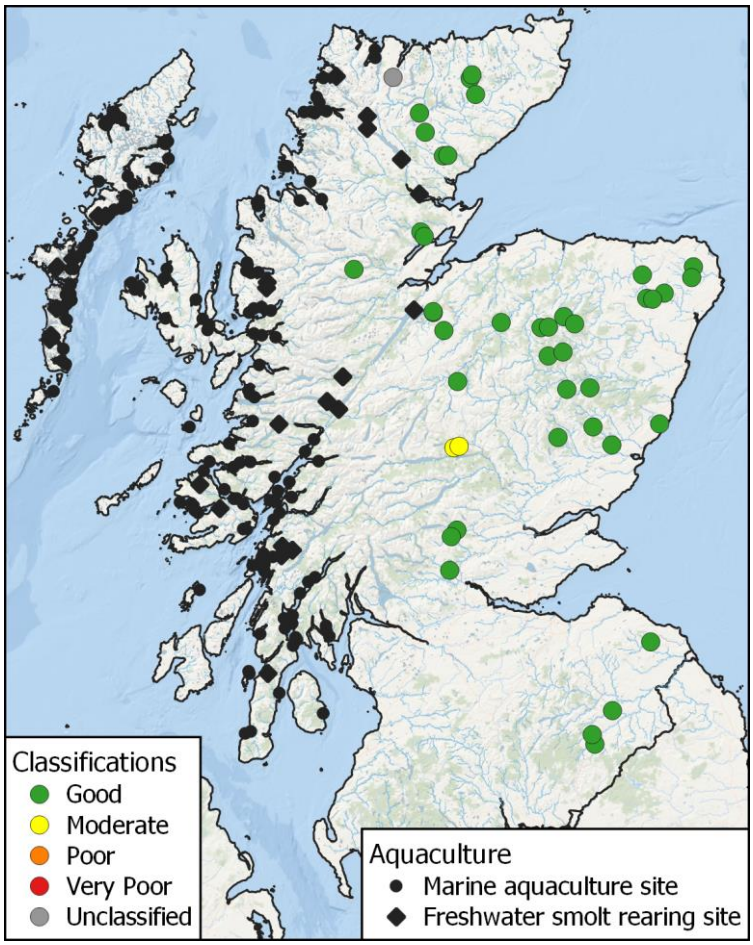

Figure 6. Genetic status classifications in the North and East regions of Scotland. Marine and freshwater aquaculture production facilities are also shown. Not included: Kyle of Sutherland, Ness & Beauly.

Classification	n	Percent
Classified	42	97.7
Unclassified	1	2.3
Level	n	Percent
Good	40	95.2
Moderate	2	4.8
Poor	0	0.0
Very Poor	0	0.0

Table 3. Summary of classification status of sample sites in the North and East regions of Scotland. Values are numbers of sites (n) and percentages of sites. Not included: Kyle of Sutherland, Ness & Beauly.

Across the large North and East region 591 fish were screened. Of the 42 sites examined, all but two adjacent sites on the Errochty Water on the upper Tay were classified as good, with no evidence of introgression (full site level details in Appendix 2). There are no marine or freshwater aquaculture facilities within this region (apart from freshwater smolt rearing facilities in the Kyle of Sutherland and Ness & Beauly, which are examined separately below), however adult escaped farm fish have been reported in rivers within this region (Walker et al., 2006). Over the entire region, no significant impacts resulting of any spawning of these fish on the genetic integrity of populations were detected in this survey.

Tay

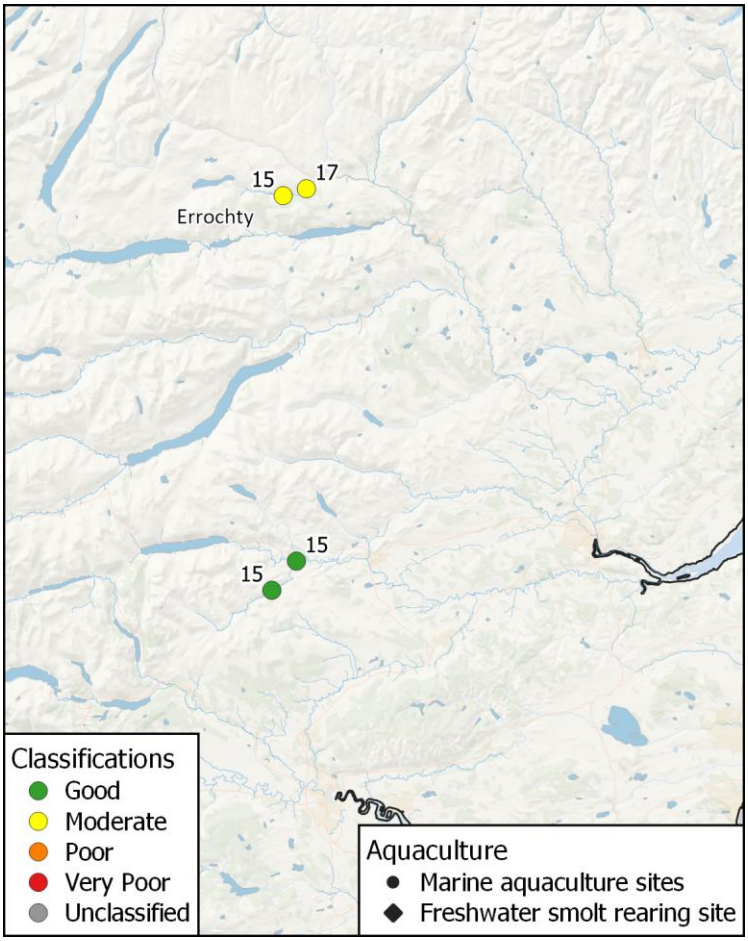

Figure 7. Genetic status classifications in the Tay region of Scotland in relation to aquaculture facilities. Numbers refer to sample numbers.

Classification	n	Percent
Classified	4	100.0
Unclassified	0	0.0
Level	**n**	**Percent**
Good	2	50.0
Moderate	2	50.0
Poor	0	0.0
Very Poor	0	0.0

Table 4. Summary of classification status of sample sites in the Tay region of Scotland. Values are numbers of sites (n) and percentages of sites.

Across the Tay region 62 fish were screened. Evidence of introgression was seen at 2 of the 4 sites classified (50%) (full site level details in Appendix 2). There are no aquaculture facilities in the Tay area and the suggestion of introgression at two of the sites on the

Errochty Water was thus unexpected. There are a number of possible reasons for this finding.

One or more farm escapees could have spawned in the upper river. This is possible given observations of farm escapees in upper river systems elsewhere (Thorstad et al., 2008; Glover et al., 2020). However, although this cannot be ruled out, this does not seem the most likely explanation given the lack of any proximate aquaculture facilities or evidence of introgression within the region more generally.

Alternatively, the results could be a statistical artefact of the analysis, the sample sizes and choice of threshold levels and tests for the colour grading system. By definition if 99% of fish are correctly evaluated, 1% will be incorrect. Across the whole North and East region (excluding the Kyle of Sutherland and Ness and Beauly systems which are influenced by freshwater smolt rearing facilities) there were 591 fish screened at 43 sites. In this region a single fish and two sites were classified as showing signs of introgression. This is well within the 99% margin of error. The significant findings at the two sites were based on the criteria relating to the identification of individual hybrid fish at one site (a single hybrid fish identified; $P(\text{wild}) = 0.739$), and at the other site there were no individual fish classified as hybrids, but the t-test comparison of $P(\text{wild})$ means to the wild reference fish was significant at the 5% level ($p = 0.020$). So, at both sites there was significant, but weak evidence of genetic change, which may be within the margin of error.

Perhaps the most likely explanation for the results is that the wild salmon in the Errochty Water may simply be genetically very distinct from surrounding sites in the eastern area due to both historic and contemporary influences, and that this distinctness has not been captured in the wild reference samples used in the analysis. This would result in the fish at this site not matching the wild reference samples and therefore result in the $P(\text{wild})$ outcomes observed at the site. Cauwelier et al. (2018) showed that the top of the large Tay system was one of the last areas of Scotland to be re-colonised after the ice sheets receded and, in that analysis, they too found samples from the upper Tay to be genetically distinct from others in the region. Initial investigation using 50 Errochty juveniles captured in 2009 and screened at 14 microsatellite markers suggests there may have been a bottleneck event in the past which could again impact the genetic signature in the tributary and increase genetic differentiation from other sites in the region (Marine Scotland, unpublished data).

The Errochty has also seen anthropogenic contemporary impacts. A section of the upper Errochty is cut-off by impassable hydroelectricity damming which also influences water flows and temperatures further down the system. There has also been significant stocking in the tributary, including directly at one of the sites (D. Summers, pers. com.). Although it has

been reported that best practice is followed during stocking, with only progeny of local wild-caught broodstock used, any stocking has the potential to impact the genetic signature of a location and increase differentiation from surrounding wild sites (Araki et al., 2007; Fraser, 2008; Araki and Schmid, 2010).

Considering the totality of the results impacting the classification of these and other sites on the east coast, it seems most likely that these sites are characterised by fish with a genetic structure that has not been captured in the reference samples used to separate wild and farmed fish. It is not, perhaps, surprising that this should be the case, considering the diverse interplay of historic and contemporary influences on salmon populations across the country.

Kyle of Sutherland

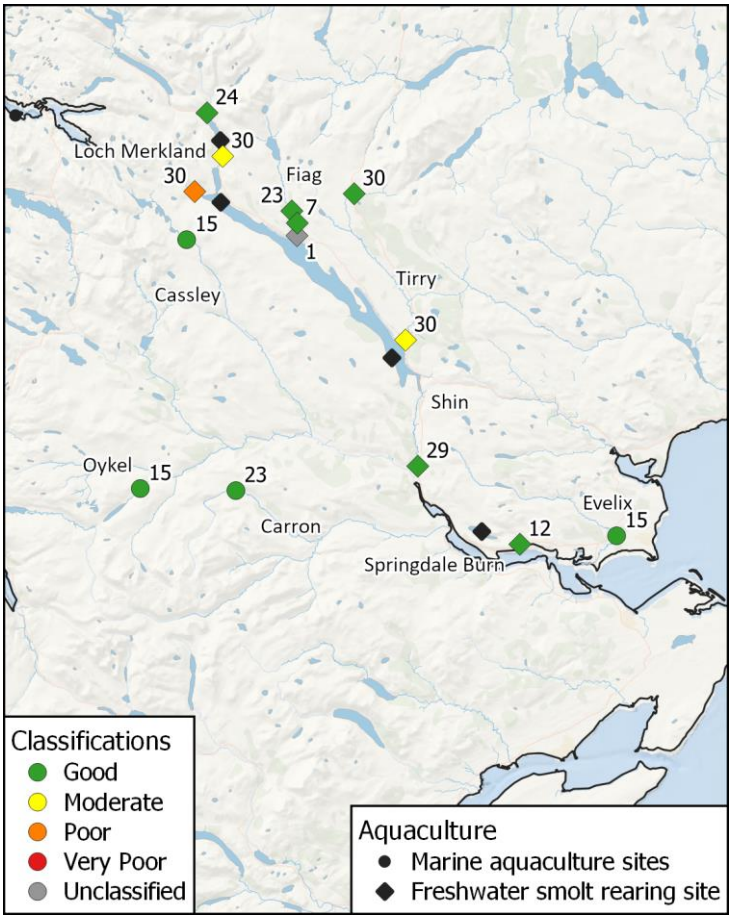

Figure 8. Genetic status classifications in the Kyle of Sutherland region of Scotland in relation to aquaculture facilities. Numbers refer to sample numbers. Sites shown as circles are NEPS sites while those shown as diamonds are supplemental sites.

Classification	n	Percent
Classified	13	92.9
Unclassified	1	7.1
Level	n	Percent
Good	10	76.9
Moderate	2	15.4
Poor	1	7.7
Very Poor	0	0.0

Table 5. Summary of classification status of Scottish sample sites in the Kyle of Sutherland region of Scotland. Values are numbers of sites (n) and percentages of sites.

Across the Kyle of Sutherland region 284 fish were screened. Evidence of introgression was seen at 3 of the 13 sites classified (23.1%) (full site level details in Appendix 2). There was no evidence of introgression on the rivers Oykel, Cassley, Carron, Evelix or Springdale Burn. Although a smolt rearing site is indicated on the Springdale Burn no fish have been present since 2010. Sites with evidence of introgression were adjacent to the active freshwater rearing locations on the Shin system. There was no evidence of introgression at sites on the Fiag, the upper Tirry, above Loch Merkland, or on the lower Shin mainstem.

Ness and Beauly

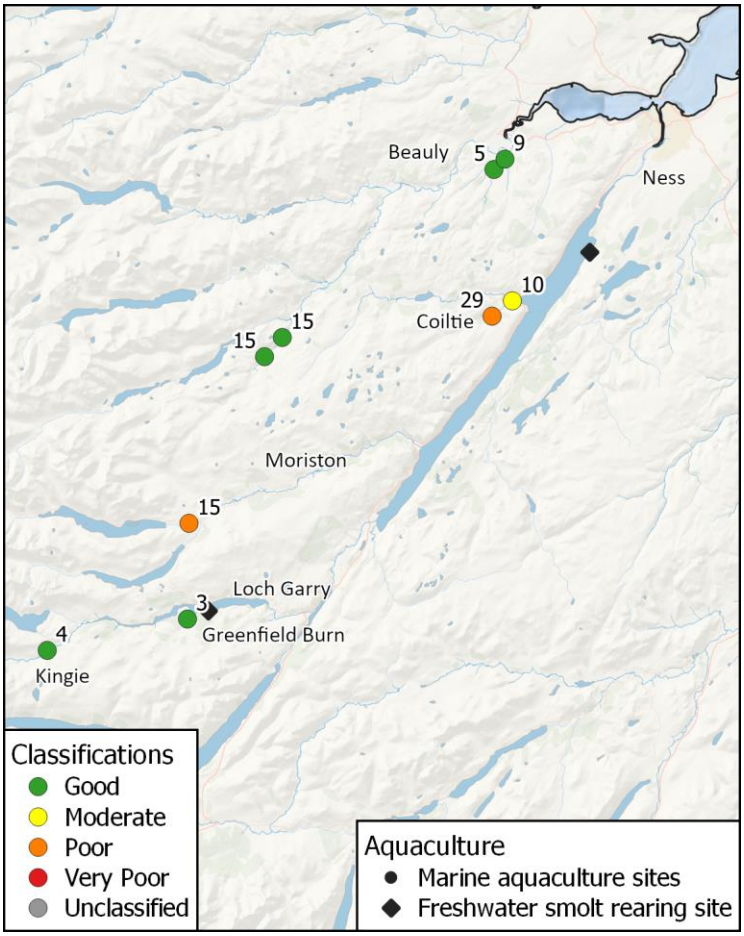

Figure 9. Genetic status classifications in the Ness and Beauly region of Scotland in relation to aquaculture facilities. Numbers refer to sample numbers.

Classification	n	Percent
Classified	9	100.0
Unclassified	0	0.0
Level	**n**	**Percent**
Good	6	66.7
Moderate	1	11.1
Poor	2	22.2
Very Poor	0	0.0

Table 6. Summary of classification status of sample sites in the Ness and Beauly region of Scotland. Values are numbers of sites (n) and percentages of sites.

Across the Ness and Beauly region 105 fish were screened. Evidence of introgression was seen at 3 of the 9 sites classified (33%) (full site level details in Appendix 2). There was no evidence of introgression in the 4 sites on the river Beauly which has no freshwater smolt

rearing facilities. However, 3 of the 5 sites on the Ness, two sites on the lower River Coiltie and one on the River Moriston, did show some evidence of introgression. Neither of the sites on the tributaries of Loch Gary (Greenfield Burn and River Kingie) showed evidence of introgression, however sample numbers here were low.

West Sutherland

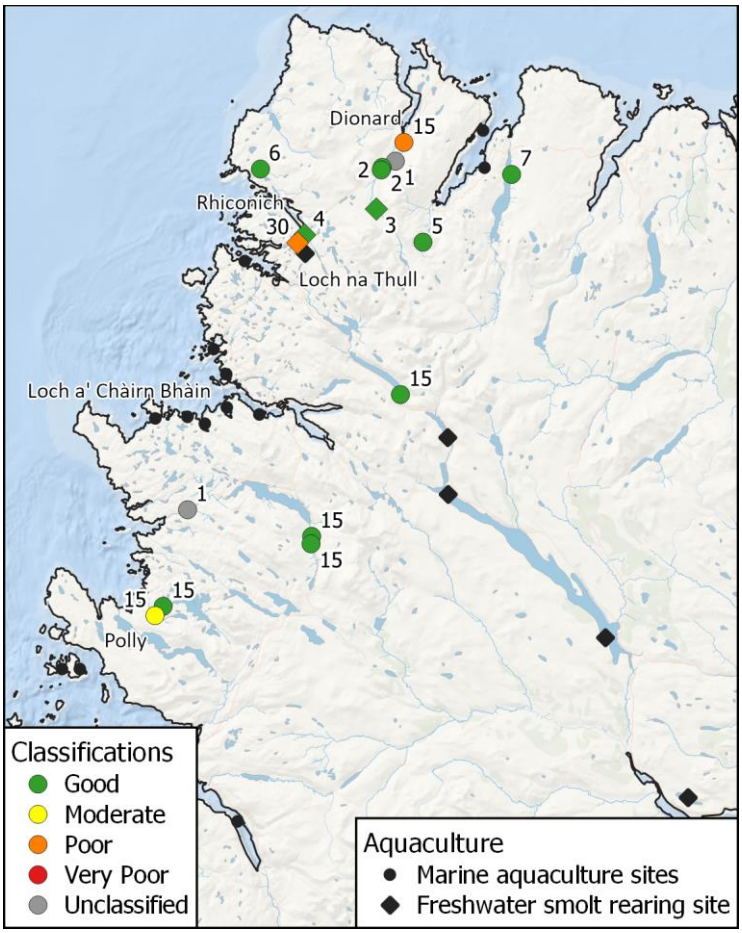

Figure 10 Genetic status classifications in the West Sutherland region of Scotland in relation to aquaculture facilities. Numbers refer to sample numbers. Sites shown as circles are NEPS sites while those shown as diamonds are supplemental sites.

Classification	n	Percent
Classified	14	87.5
Unclassified	2	12.5
Level	n	Percent
Good	11	78.6
Moderate	1	7.1
Poor	2	14.3
Very Poor	0	0.0

Table 7. Summary of classification status of sample sites in the West Sutherland region of Scotland. Values are numbers of sites (n) and percentages of sites.

Across West Sutherland 151 fish were screened. Evidence of introgression was seen at 3 of the 14 sites classified (21.4%) (full site level details in Appendix 2). Within the region there is a single freshwater smolt rearing facility in Loch na Thull and the sample site immediately downstream of this was classified as Poor. In the adjacent site on the Rhiconich river there was no evidence of introgression (although only 4 fish were examined). The most northern and most southern sites in the region, situated on the Dionard and Polly, were both classified as showing signs of introgression. On both of these river systems it was the lowest site in the system (nearest river mouth) that showed genetic changes, with the site on the lower point of the Dionard classified as Poor and the site on the lower Polly as Moderate. No sites were sampled in the immediate area surrounding Loch a' Chàirn Bhàin, that has the highest marine aquaculture production concentration in the area.

Skye and Wester Ross

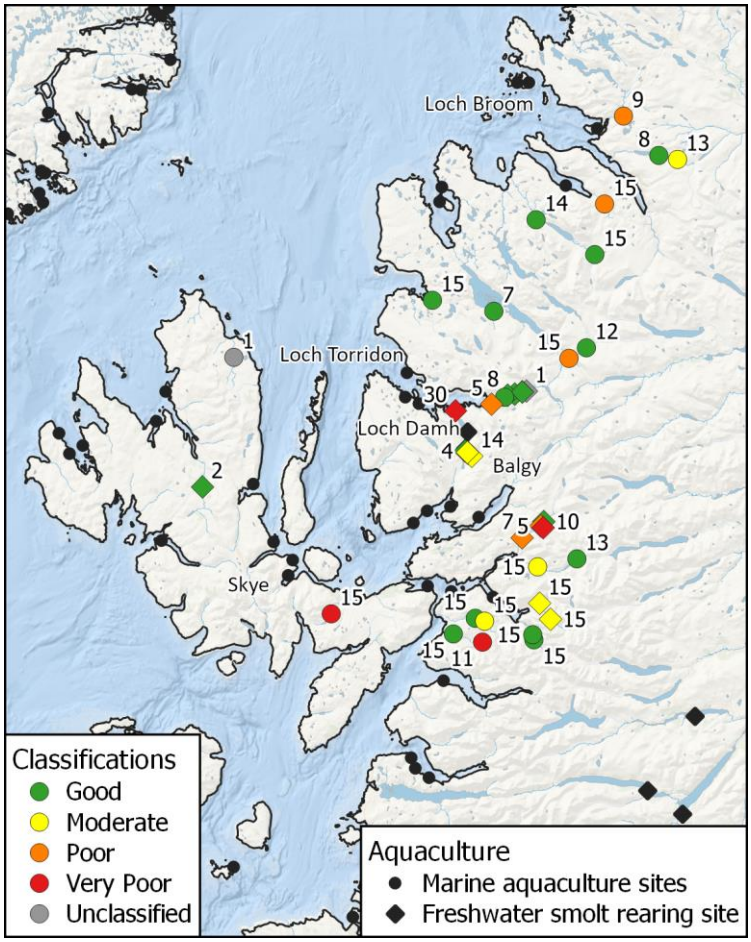

Figure 11. Genetic status classifications in the Sky and Wester Ross region of Scotland in relation to aquaculture facilities. Numbers refer to sample numbers. Sites shown as circles are NEPS sites while those shown as diamonds are supplemental sites.

Classification	n	Percent
Classified	37	94.9
Unclassified	2	5.1
Level	**n**	**Percent**
Good	19	51.4
Moderate	8	21.6
Poor	6	16.2
Very Poor	4	10.8

Table 8. Summary of classification status of sample sites in the Sky and Wester Ross region of Scotland. Values are numbers of sites (n) and percentages of sites.

Across the Skye and Wester Ross region 487 fish were screened. Evidence of introgression was seen at 18 of the 37 sites classified (48.6%) (full site level details in Appendix 2).

Classification of sites showed genetic changes across most of the Wester Ross area. Three clusters of introgression were seen around the sea lochs of Broom and Torridon, and the lochs directly east of Skye. These contain some of the highest concentrations of marine aquaculture facilities in the area. There are also freshwater smolt rearing sites in Loch Damh on the river Balgy with sites on this system having Moderate (upstream of the loch) to Very Poor (downstream of the loch) classifications.

Lochaber

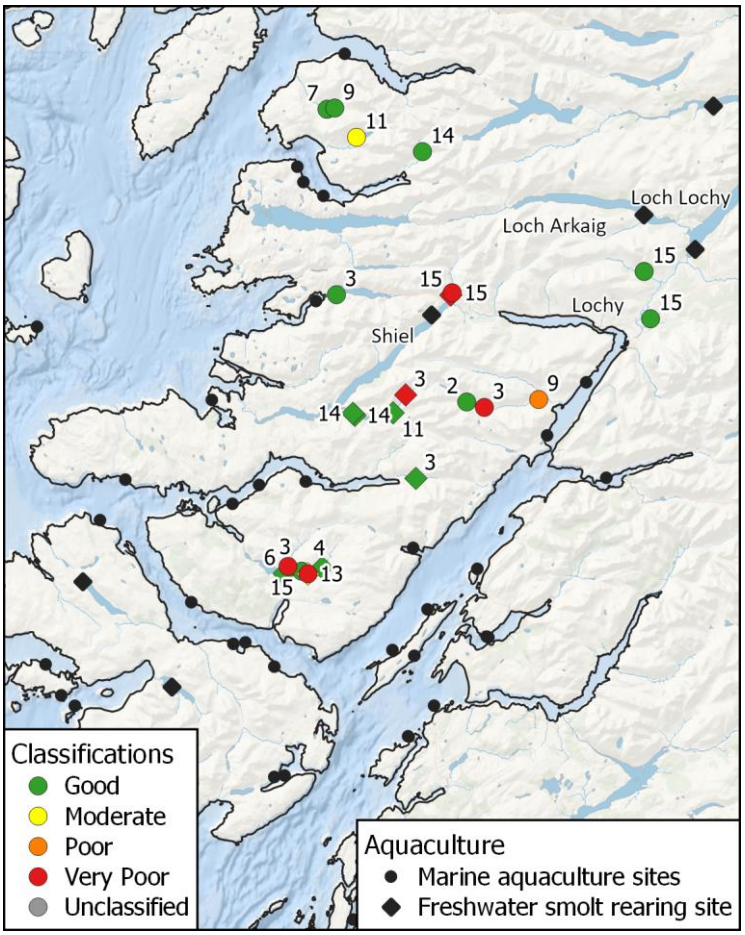

Figure 12. Genetic status classifications in the Lochaber region of Scotland in relation to aquaculture facilities. Numbers refer to sample numbers. Sites shown as circles are NEPS sites while those shown as diamonds are supplemental sites.

Classification	n	Percent
Classified	23	100.0
Unclassified	0	0.0
Level	n	Percent
Good	14	60.9
Moderate	1	8.7
Poor	2	4.3
Very Poor	6	26.1

Table 9. Summary of classification status of sample sites in the Lochaber region of Scotland. Values are numbers of sites (n) and percentages of sites.

Across Lochaber 219 fish were screened. Evidence of introgression was seen at 9 of the 23 sites classified (34.8%) (full site level details in Appendix 2) and these were located across the region. There are freshwater smolt rearing sites in the area. In the Lochy system there are rearing sites on Lochs Lochy and Arkaig. However, the two sample sites downstream of these lochs showed no evidence of introgression. The situation was different in the Shiel system, with the two sites immediately adjacent to the smolt rearing facility being classified as Very Poor.

Argyll

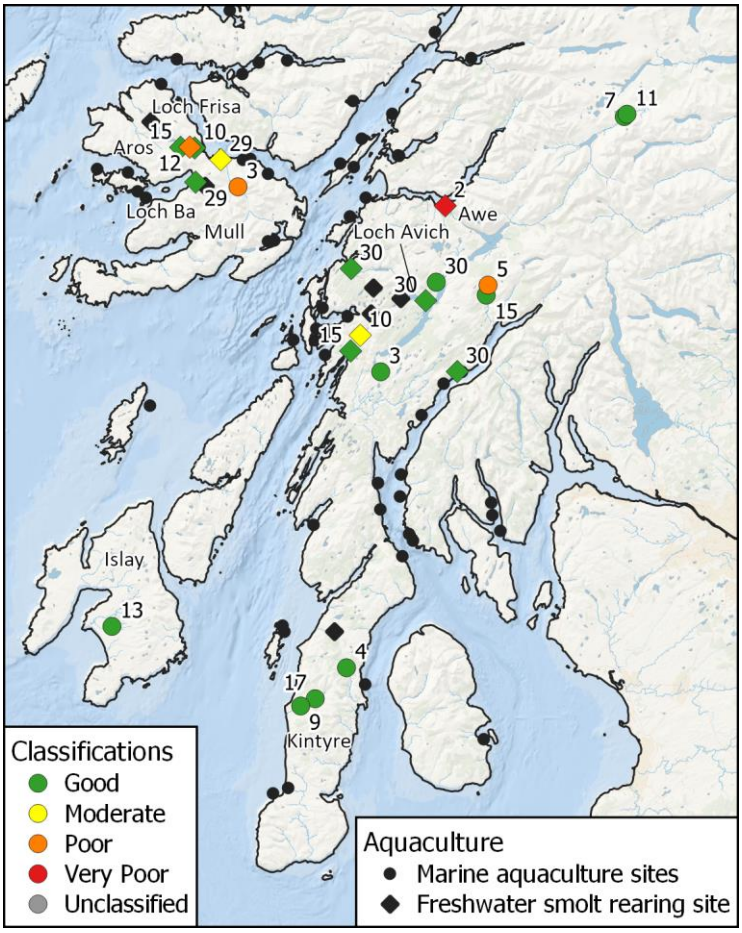

Figure 13. Genetic status classifications in the Argyll region of Scotland in relation to aquaculture facilities. Numbers refer to sample numbers. Sites shown as circles are NEPS sites while those shown as diamonds are supplemental sites.

Classification	n	Percent
Classified	22	100.0
Unclassified	0	0.0
Level	**n**	**Percent**
Good	16	72.7
Moderate	2	9.1
Poor	3	13.6
Very Poor	1	4.5

Table 10. Summary of classification status of sample sites in the Argyll region of Scotland. Values are numbers of sites (n) and percentages of sites.

Across Argyll 329 fish were screened. Evidence of introgression was seen at 6 of the 22 sites classified (27.3%) (full site level details in Appendix 2). These sites were in the centre

of the region and on the Isle of Mull but not on the Isle of Islay or the Kintyre peninsula (although sampling is limited in these locations). There are also a number of freshwater smolt rearing facilities in the region with a mixed pattern of introgression at the sample sites within the same systems. In the River Awe system, the two sites closest to the smolt rearing facility on Loch Avich had no evidence of introgression, however, the site near to the river mouth was classified as Very Poor. On the Isle of Mull one of the three sites on the River Aros, situated below the smolt rearing facility on Loch Frisa showed evidence of introgression while the other two did not. There was also no evidence of introgression below the rearing facility on Loch Ba.

Clyde

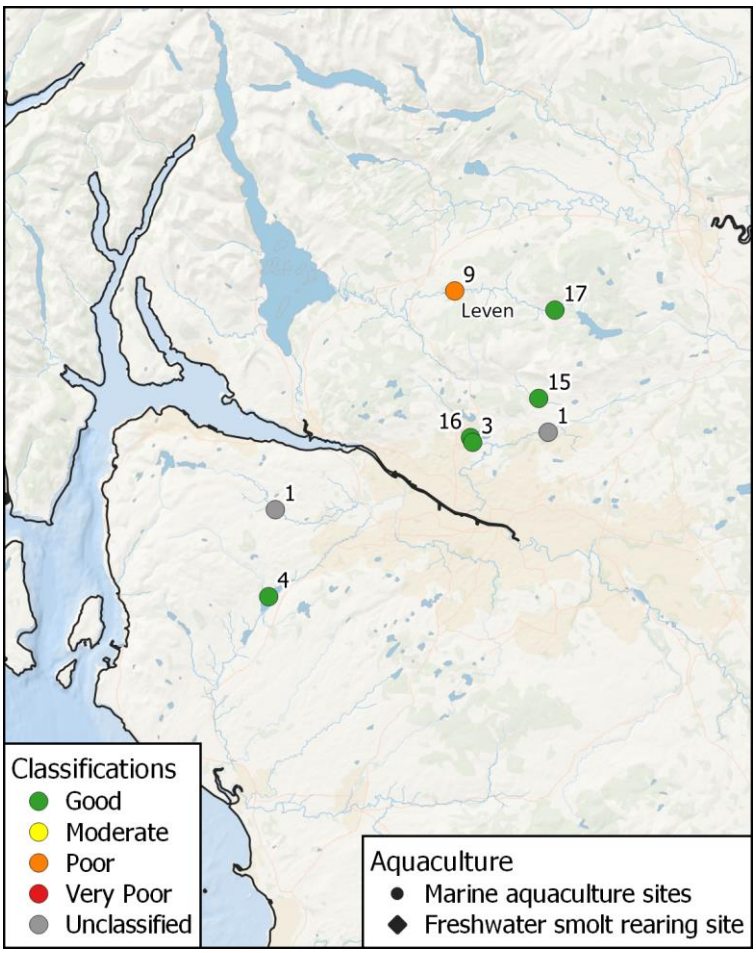

Figure 14. Genetic status classifications in the in the Clyde region of Scotland in relation to aquaculture facilities. Numbers refer to sample numbers.

Classification	n	Percent
Classified	6	75.0
Unclassified	2	25.0
Level	n	Percent
Good	5	83.3
Moderate	0	0.0
Poor	1	16.7
Very Poor	0	0.0

Table 11. Summary of classification status of sample sites in the Clyde region of Scotland. Values are numbers of sites (n) and percentages of sites.

Across the Clyde 66 fish were screened. Evidence of introgression was seen at 1 of the 6 sites classified (16.7%) (full site level details in Appendix 2). The single site with evidence of introgression was on the lower River Leven, with a second site further up this system showing no evidence of introgression. Sampling was limited in the rest of the region, but of the sites screened all were classified as Good.

Southwest

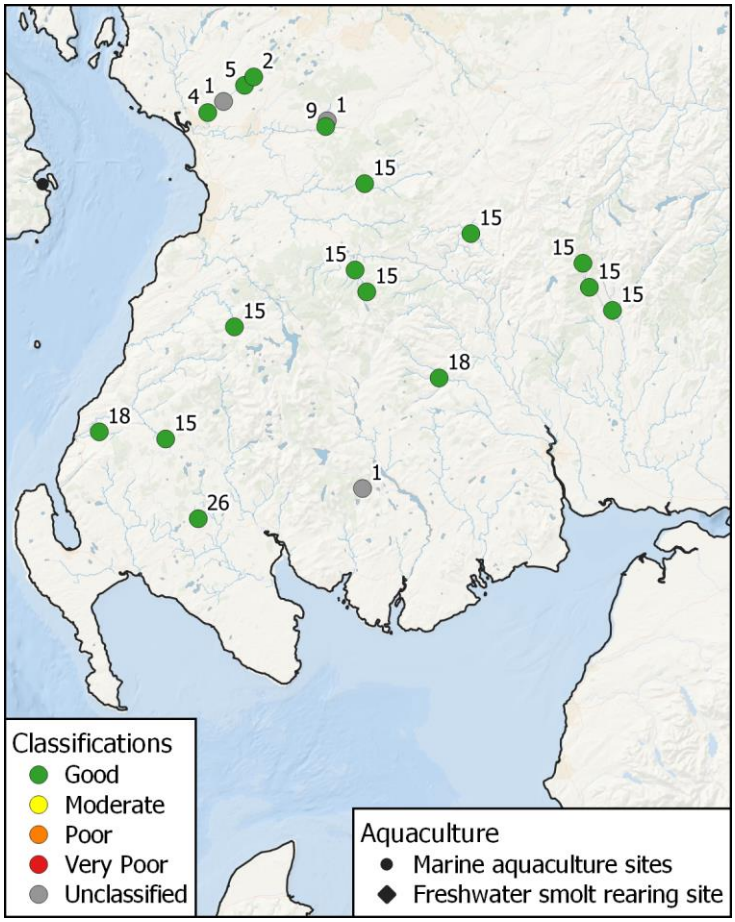

Figure 15. Genetic status classifications in the in the Southwest region of Scotland in relation to aquaculture facilities. Numbers refer to sample numbers.

Classification	n	Percent
Classified	16	84.2
Unclassified	3	15.8
Level	**n**	**Percent**
Good	16	100.0
Moderate	0	0.0
Poor	0	0.0
Very Poor	0	0.0

Table 12 Summary of classification status of sample sites in the Southwest region of Scotland. Values are numbers of sites (n) and percentages of sites.

Across the Southwest region 220 fish were screened. No evidence of introgression was seen at any of the 16 sites classified (full site level details in Appendix 2). There are no

marine or freshwater aquaculture facilities in the immediate vicinity of the sites in this region, and no evidence of introgression was detected at the sites examined.

Hebrides

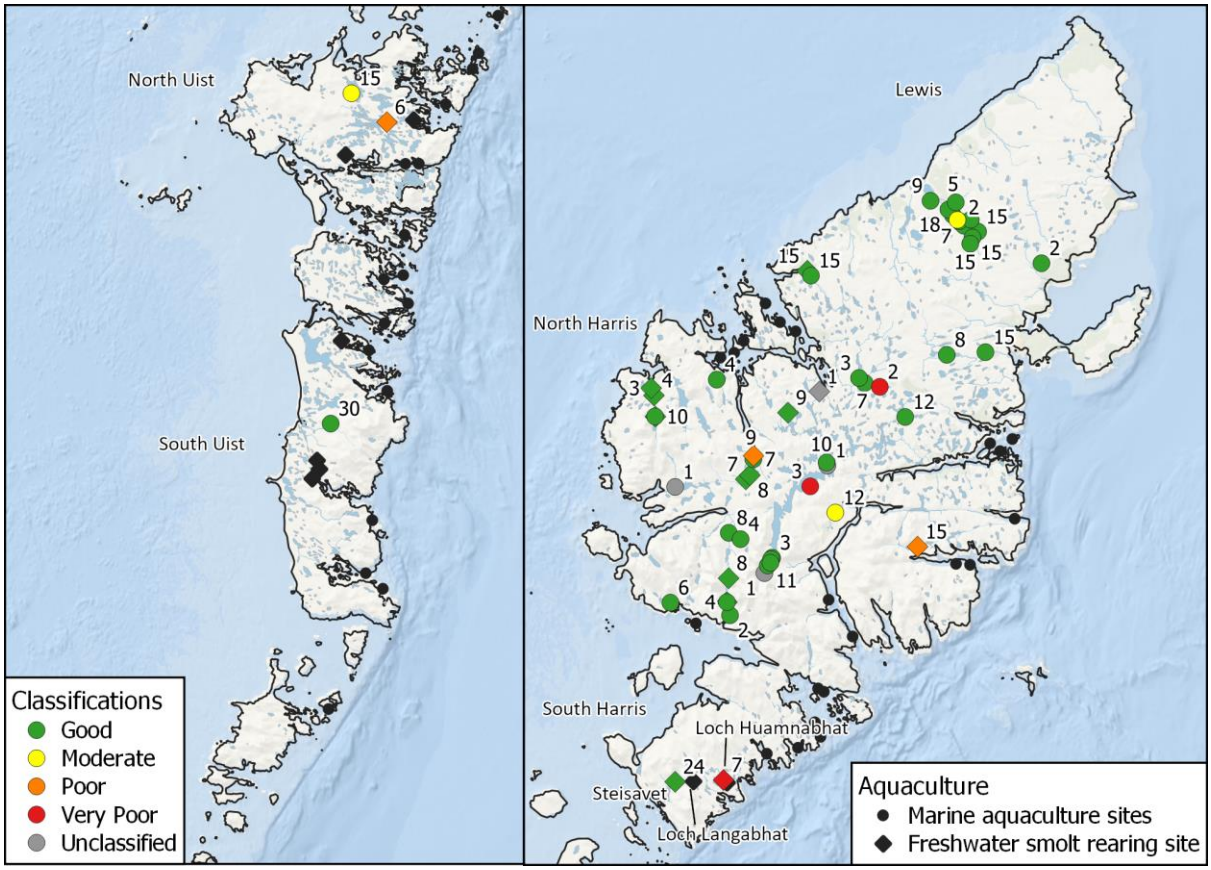

Figure 16. Genetic status classifications in the Outer Hebridean region of Scotland in relation to aquaculture facilities. Numbers refer to sample numbers. Sites shown as circles are NEPS sites while those shown as diamonds are supplemental sites.

Classification	n	Percent
Classified	51	92.7
Unclassified	4	7.3
Level	**n**	**Percent**
Good	42	82.4
Moderate	3	5.9
Poor	3	5.9
Very Poor	3	5.9

Table 13. Summary of classification status of sample sites in the Outer Hebridean region of Scotland. Values are numbers of sites (n) and percentages of sites.

Across the Outer Hebrides 485 fish were screened. Evidence of introgression was seen at 9 of the 51 sites classified (17.6%) (full site level details in Appendix 2). The single site examined on South Uist was classified as Good, but both sites on North Uist showed evidence of introgression. On South Harris, the site on the Steisavet, downstream of the freshwater smolt rearing facility on Loch Langabhat had no evidence of introgression, whereas the site adjacent to the smolt rearing facility on Loch Huamnabhat was classified as Very Poor. All North Harris sites were classified as good. On Lewis, most sites were classified as Good. However, there were sites with evidence of introgression throughout the area with no obvious pattern.

Shetland

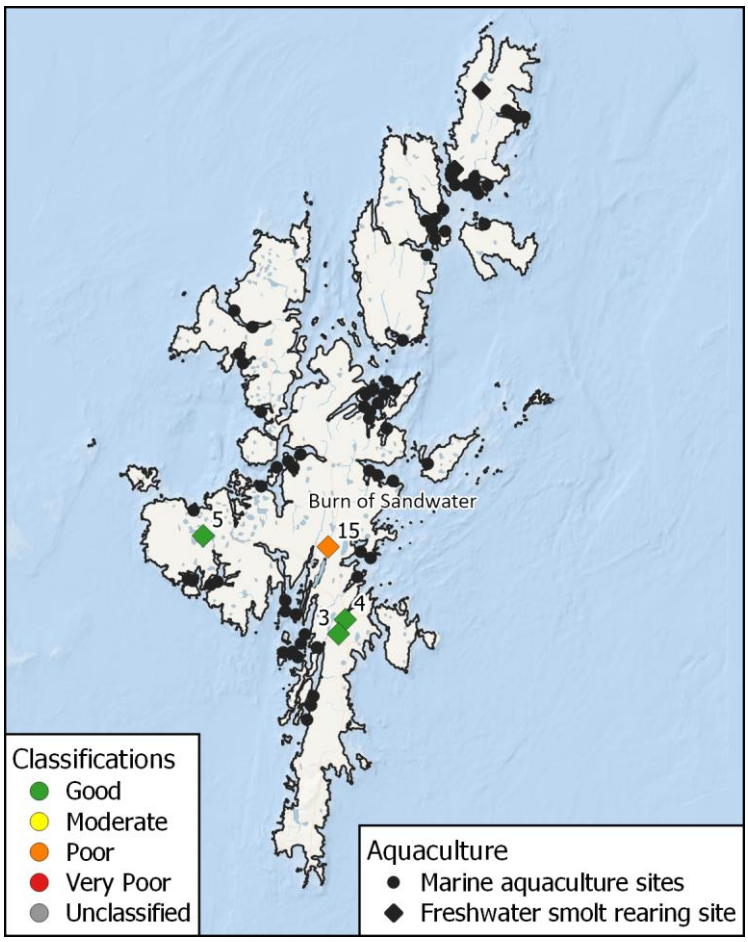

Figure 17. Genetic status classifications in the Shetland region of Scotland in relation to aquaculture facilities. Numbers refer to sample numbers. Sites shown as circles are NEPS sites while those shown as diamonds are supplemental sites.

Classification	n	Percent
Classified	4	100.0
Unclassified	0	0.0
Level	n	Percent
Good	3	75.0
Moderate	0	0.0
Poor	1	25.0
Very Poor	0	0.0

Table 14. Summary of classification status of sample sites in the Shetland region of Scotland. Values are numbers of sites (n) and percentages of sites.

Across Shetland 27 fish were screened. Evidence of introgression was seen at 1 of the 4 sites classified (25.0%) (full site level details in Appendix 2). Sampling and fish numbers were limited in Shetland, however the site on the Burn of Sandwater with the most fish screened (15) showed evidence of introgression.

Summary of findings

Escapees of Norwegian origin salmon from aquaculture production facilities in Scotland have introduced genetic material from farm strains into some wild populations. This introgression is not uniform across the country and appears to be concentrated near areas of marine aquaculture production and freshwater smolt rearing. Outside of these areas any evidence for interbreeding was very limited.

Introgression associated with marine aquaculture

It is clear that introgression has occurred in salmon populations in areas associated with marine aquaculture production. The Shetland Islands, the Hebrides and the entire mainland west coast down to the river Clyde are affected. A substantial number of sites within these regions show evidence of introgression, however, there are also adjacent sites within these regions that appear to have retained their genetic integrity. Such a patchwork is very similar to that observed in Norway where, even in the most highly affected areas, sites can be found with no detectable introgression (Diserud et al., 2020). Further work is necessary to understand these observations and identify controls on the observed spatial patterns (e.g. Mahlum et al., 2021). Nevertheless, it is clear that salmon aquaculture is associated with the introgression of genetic material from Norwegian origin farm fish into wild populations in the areas of production.

It is striking that in the area south of marine aquaculture production on the west coast and on the Scottish north coast immediately north/northeast of production in Loch Eriboll there was no evidence for introgression. In addition, while escaped farm fish have been detected in rivers in these areas, and further afield, no genetic changes were detected. There are a number of reasons that may influence this observation related to conditions within both the freshwater and marine environments. The abundance of escaped fish in rivers and the levels of introgression resulting from this have both been found to be strongly correlated with the aquaculture intensity in the immediate vicinity (Diserud et al., 2020; Mahlum et al., 2021), and the pattern of introgression observed here matches that finding. The abundance of wild salmon, mean annual discharge, and the interaction between sea loch placement and wild salmon abundance are also important predictors of the escapee abundance in rivers (Mahlum et al., 2021) and this may also explain some of the intra-regional variations found on the west coast and Hebridean islands. Generally bigger rivers which may have larger wild populations within the southwest region may mean they are also less susceptible to impacts as has been reported elsewhere (Wringe et al., 2018). It may also be that escaped fish are poorly adapted to the acidified river environments found in certain areas of this region (Prodöhl et al., 2019). Further work will be necessary to examine the determinants of the spatial patterns seen within the west coast and Hebrides.

The lack of introgression detected immediately to the south and northeast of the aquaculture production areas on the west of mainland Scotland may also be influenced by the current patterns within this area and the movements of farmed fish post-escape.

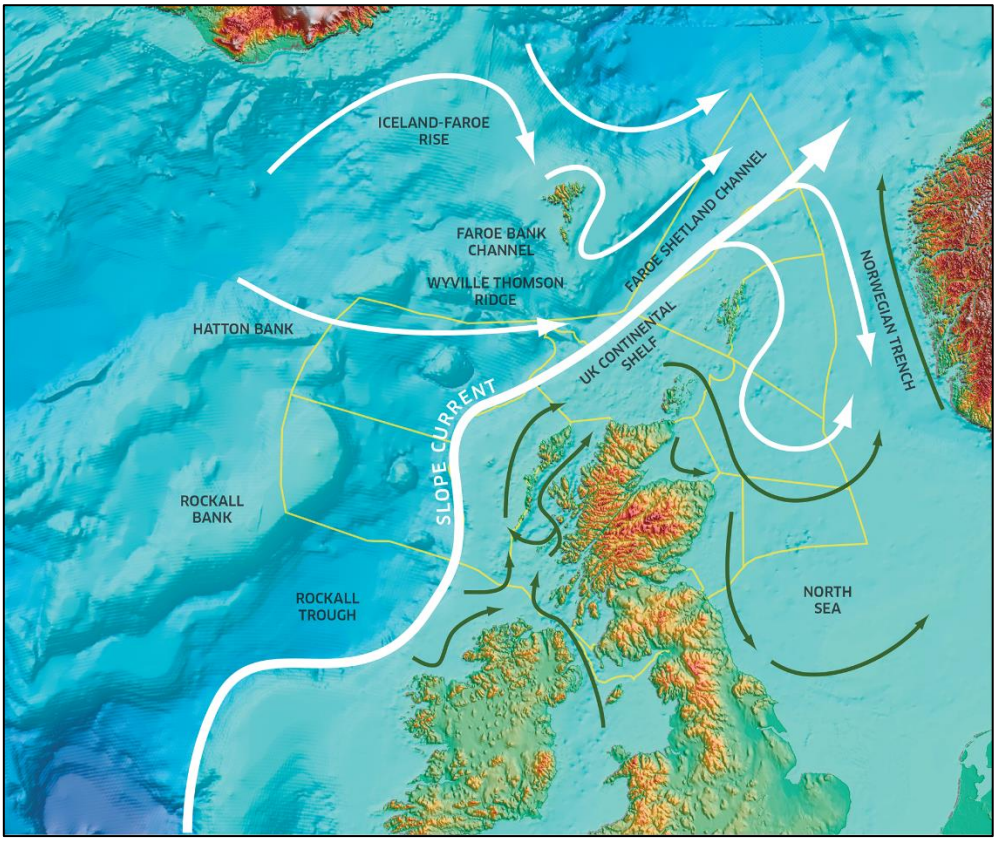

Figure 18. Circulation map representing the general circulation pattern within the North Atlantic and North Sea areas. It should be noted that flow is not confined to these arrow tracks. Circulation of Atlantic water is shown by the white arrows, with coastal circulation represented by the green arrows. The outline of the Scottish Marine Regions and Offshore Marine Regions are shown by the yellow lines. From https://marine.gov.scot/sma/assessment/circulation

Feeding areas in their first year for eastern Atlantic salmon from Scotland are off the west coast of Norway in the Norwegian sea (Gilbey et al., 2021). In order to reach these feeding areas wild fish from the Scottish west coast perform a northwards migration which follows, and is largely driven by, the prevailing currents in this area (Dadswell et al., 2010; Ounsley et al., 2019; Gilbey et al., 2021). It is perhaps unsurprising that fish escaping at a relatively early age will tend, in the absence of other cues, to follow these same currents. Further, the natural migratory tendency of wild salmon is disrupted in farmed fish and simulated escape events have shown that released farm salmon appeared to move with the current and have a very weak homing instinct (Hansen, 2006). This would explain the lack of fish seen in areas to the south and northeast of the production areas. It is suggested that salmon that escape during early autumn the year before they become sexually mature are transported with the currents to more northerly areas and subsequently these fish have low survival

(Hansen, 2006) and/or may enter rivers far from their escape location (Hansen and Youngson, 2010). Larger salmon escaping when nearer maturity may enter rivers relatively quickly and these tend to enter rivers closest to their escape point (Wringe et al., 2018). Such a mechanism is supported by the close relationship between numbers of escapees in rivers and aquaculture intensity in an area (Mahlum et al., 2021) and also reports of large numbers of fish being observed by anglers and members of the public following large scale escapes.

Overall, the levels of introgression detected here would suggest that interactions between aquaculture location, prevailing currents and the size and health of the wild population are all factors likely to be driving the observed spatial patterns. Work is underway to disentangle these influences.

Introgression associated with freshwater smolt rearing

Escapees from freshwater smolt rearing facilities can lead to hybridisation and introgression in the systems where such production takes place. Such events appear evident in the large North and East region where there is no marine aquaculture production, and where (apart from the outlier Errochty) the only two systems with notable levels of genetic change were the Shin and Ness; the only systems where farmed smolt production takes place. As with the spatial patterns of impacted populations observed in relation with marine farms, however, not every situation where there was freshwater smolt production resulted in detectable levels of genetic change. Again, the controls on these spatial patterns require investigation. Factors such as aquaculture fish security measures, distance to the facilities, size and health of the wild populations would be likely determinants. The availability and suitability of juvenile habitat may also be a factor. It is notable that during the design of the ad hoc part of the sampling programme, sample sites were targeted to tributaries around the smolt rearing facilities., However, in a number of cases, collection of fish from these sites was not possible because due to a reported lack of juvenile habitat. In such situations there is no wild fish population for any escapees to interbreed with.

Genetic composition of sites showing evidence of introgression

Examination of the individual fish *P*(wild) values at sites showing evidence of introgression allows the composition of the various hybrid types to be examined (Figure 19). Firstly, it can be seen that, as expected, the 1-percentile cut-off level used is very conservative. When all fish are examined, there are many with *P*(wild) values at or immediately around one. These are classified as pure wild fish. There are also a small number with *P*(wild) values just less than one which likely represent the lower tail of the pure wild *P*(wild) distribution, or perhaps

very late generation hybrids with remnants of farmed genomic composition. Taking these results into consideration, in future, the cut-off value could be reasonable redefined at just below this level (i.e. around 0.9) considering the remainder of the *P*(wild) values observed. Work is underway to better define this cut-off in light of the insights gained from the analysis of the data obtained for this investigation.

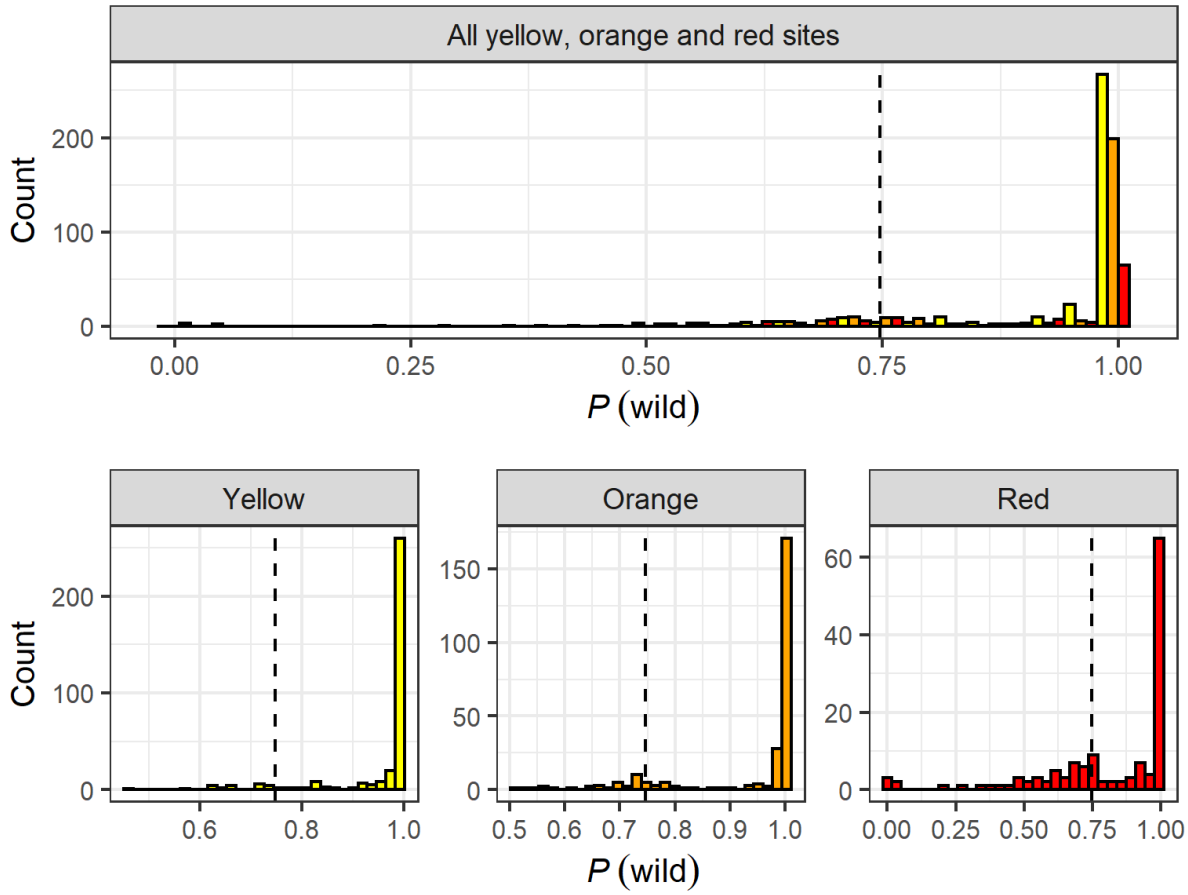

Figure 19. *P*(wild) levels of all individual fish at sites with evidence of introgression and classified as yellow, orange or red. Vertical dashed line represents the 1-percentile P(wild) cut-off of 0.747.

Together with the pure wild fish, both pure farmed fish and hybrid farm/wild fish can be observed in the data. Pure farm fish can be seen in the red site individual data (Figure 19). These fish have *P*(wild) values around zero. There were five such fish from three sites which are all located in Lochaber (Figure 20).

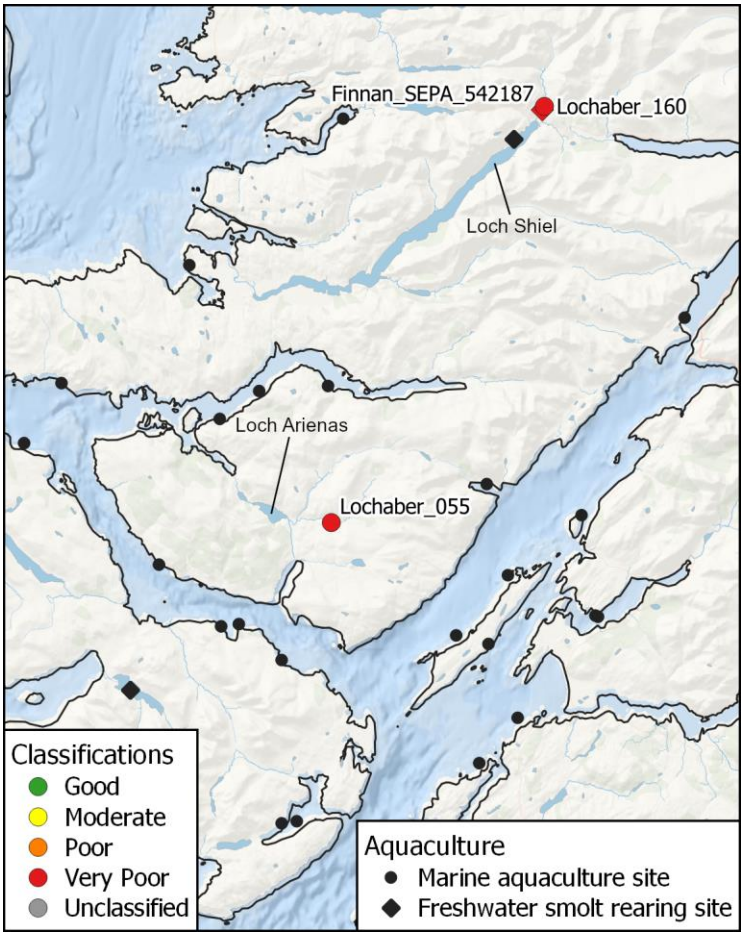

Figure 20. Sites where pure farm juveniles were detected.

Two of the sites (Finnan_SEPA_542187 and Lochaber_160) each had one pure farm fish and these sites were immediately upstream of the freshwater smolt rearing aquaculture facility on Loch Shiel. At the other site (Lochaber_055) three pure farm fish were detected. This site is on a tributary of the River Aline system and on this same system to the west of the site is Loch Arienas. This loch had no freshwater smolt sites that were classified as active in 2018 or 2019 when sampling was undertaken. However, there was previously an active freshwater smolt rearing site on this loch which ceased operation in 2017 (Lochaber Fisheries Trust, 2017; Marine Scotland data). It is thus again clear, as has previously been observed (Gilbey et al., 2018a), that juvenile escapees occur in areas with freshwater smolt rearing, and that these fish can be detected in the wild using the approaches utilised here.

In addition to the pure wild and pure farmed fish, there is also evidence of hybrids within the sites (Figure 19). These fish are seen to have *P*(wild) values which are intermediate to the pure farm and wild fish. The *P*(wild) values of these fish suggest a complex mixing pattern of hybridisation has occurred which may have covered a number of generations. F1 fish would be expected to have *P*(wild) values around 0.5 and such fish are seen here. BC1W would be expected to have *P*(wild) values around 0.75 and again such fish are observed. Fish with

P(wild) values of 0.75 could also have arisen from crossing between pure farmed and BC1F fish and so it is difficult to specify exactly the proportions of each hybrid type observed.

Over all the sites there is a distribution of *P*(wild) values suggesting hybrid fish of multiple generations. The majority of the hybrids identified have *P*(wild) between 0.5 – 0.75 which suggests recent hybridisation events impacting the last few generations are driving the patterns observed. However, a continuous range of *P*(wild) values are also seen suggesting complex mixing of hybrids types over a number of generations. Distributions of all the types overlap and so it is difficult to identify the hybrid types directly. Work is underway with the data produced in this investigation to better define the various hybrid groups observed.

The range of *P*(wild) values observed in the hybrid fish would suggest the majority are from relatively recent hybridisation events. This observation means that it is very unlikely that the pattern of introgression detected is primarily driven by inputs of farmed fish from historical stocking from multiple generations in the past. It does not, however, mean that historic introgression may still not still be influencing the sampled populations. There are fish with a range of *P*(wild) which is indicative of multiple generations of complex mixing. This pattern is likely driven by more recent hybridisation events, but may also incorporate older patterns of mixing. It is also the case that selection on fitness traits will not influence all genes equally, and while some of the introgression signal associated with the genome of farmed fish may be quickly removed by such selection, some genes from historical stocking may persist for long periods if their influence is small individually (Castellani et al., 2018). Cumulatively these impacts may be significant over the long term (McGinnity et al., 2003; Castellani et al., 2018). Further, genes which may actually be beneficial (e.g. immunological genes) may face a positive selection pressure in the wild. The samples and data collected here will allow these influences to start to be disentangled.

Discussion

The data analysed here clearly show there has been hybridisation and introgression of genetic material of Norwegian aquaculture strains into Scottish wild salmon populations associated with areas of marine or freshwater production. The regional differences in levels of introgression are striking, and are usually associated with the presence of marine or freshwater production in the immediate area/system. Previous studies indicate there are significant negative fitness consequences of farm/wild hybridisation and introgression of aquaculture origin genes into wild populations (Castellani et al., 2018; Sylvester et al., 2019 and references within these). However, the degree to which these impacts are influencing wild salmon population health in Scotland is not yet known. Integrated studies are now

underway within Marine Scotland Science, using both NEPS and the survey results presented here, to try to quantify more fully the prevalence and extent of any impacts.

Future research directions

This study has identified site-wise variability in the genetic integrity of wild salmon populations with respect to potential interbreeding with salmon of Norwegian origin across Scotland. However, with the exception of observations around the location of local aquaculture production, it has not yet identified the other factors controlling the spatial variability in levels of introgression. Additionally, it was not possible to provide a statistically balanced quantitative assessment of levels of introgression on salmon populations at a regional level. Future work will be required with developments in spatial regression modelling and the formal NEPS GRTS design to answer these questions. Improved characterisation and understanding of spatial patterns of the genetic status of salmon populations have the potential to inform management of current and future interactions between wild and farmed fish. Nevertheless, the study provides a preliminary baseline of the geographic spread of introgression as a snap-shot in time.

Obtaining a quantitative unbiased estimate of regional patterns of introgression

The site-level overview presented here reports observed introgression on a site-wise basis. However, because the sample sites represent a statistical sample drawn as part of the NEPS GRTS design these results can be combined to give quantitative estimates of regional patterns of introgression in salmon populations. Such work is planned within Marine Scotland Science. With repeat surveys over time, it would also be possible to understand regional trends and to provide a quantified balanced baseline from which to assess particular large escape events.

Identifying optimum site and individual measures of genetic status

A number of different statistical approaches were used here to classify individual fish and sites. To allow comparison with previous studies, the site-level methods largely followed those employed in Norway, although sample sizes indicate that results should be treated with appropriate caution in some cases. However, in contrast to the Norwegian situation it is possible to identify individual hybrid fish with considerably greater confidence in Scotland due to the substantial genetic differences between Norwegian origin farm strains and wild Scottish fish. Individual identification based on *P*(wild) values may thus be considered extremely robust in the Scottish context and it may be that in future analysis this metric

should be the focus for the individual and site levels if sample sizes remain the same. Further analysis will help inform the best metric to be employed in such surveys in Scotland.

Environmental controls on the spatial distribution of site classification

Even in regions characterised by substantial introgression and rivers in these regions with sites categorised as very poor there were neighbouring sites where no introgression was detected, a situation that has also been observed in Norway. Using recent developments in spatial regression modelling it may be possible to investigate environmental controls on the spatial variability of the site classifications as the available data on introgression and sample sizes grow over time. Factors including distance to fish farm sites and any history of escapement, density of marine sites in an area, river size/flow characteristics, marine geography/bathymetry characteristics, distance upstream, population size, and population health can all be integrated and impacts modelled to identify major determinants of classification.

Prediction of the genetic status of a population

Once models have been developed to understand and explain spatial variability in genetic status it is possible that these same models could also be used to predict the effects of new aquaculture sites, and/or changes in current site locations and densities. Assuming that useful models can be developed, these could be used to inform future planning decisions on the sustainable development of the industry while at the same time minimising impacts on wild populations.

Inclusion of hybrid fish in wild reference samples

The wild reference samples used in the current study were obtained from fish chosen to capture, as much as possible, the wild genetic structure of Scottish salmon populations. However, unlike in Norway, where historic pre-aquaculture samples were available, the samples used here were contemporary ones. As such, and especially in areas considered at risk of introgression, there may have been inadvertent inclusion of farm/wild hybrid fish. A number of obvious hybrid F1 fish were screened out of the reference samples at an early stage (see Gilbey et al., Submitted), however it is likely that some later generation fish were still included. Such inclusion would tend to make the two reference groups (farm and wild) more similar and slightly reduce the power to detect true hybrid fish. The results are thus conservative in their overall findings i.e. they may under-estimate the true levels of introgression. However, even if a small number of such fish are included in the original reference samples, the creation of the *in silico* (computer generated offspring) reference

centroids to which the wild fish were compared would tend to negate hybrid influence. Creation of these centroids was achieved by *in silico* random mating within the reference groups. As such the impact of any hybrids in the reference group will have been diluted due to the inclusion of many non-hybrid fish from around the country. Work is required to quantify impacts from inclusion of hybrid fish in the reference dataset.

Age-class specific mortality

Differential mortality of hybrid compared to wild fish means that the proportions of hybrid fish will change as a cohort ages from egg to adult (Sylvester et al., 2019). These changes have not yet been properly quantified in Scotland. Where different age classes of the same cohort are available (i.e. in repeatedly visited NEPS sites) this could be examined assuming that differences between repeat observations relate to differential mortality rather than emigration. It would be useful to quantify this effect and so allow better integration of data from all age classes. In order to include eggs, fry and adults, further collections would be required over and above those collected under NEPS so far using a bespoke sampling protocol.

Size and growth effects

Farm/wild hybrid juvenile fish may exhibit different growth rates when compared to the pure wild type (McGinnity et al., 1997; Glover et al., 2017 and references therein). Phenotypic data collected as part of NEPS can be integrated with the hybridisation data presented here to examine such effects in the wild in Scotland. Such growth differences are important, as they may influence a number of traits including competitive behaviour, size at age, age at emigration and timing of emigration, so potentially changing the character of the population.

Catchability

Differences in growth, aggression, dominance and risk avoidance have been observed in farm, hybrid and wild fish under natural conditions (Glover et al., 2017 and references therein). Such differences in behaviour have the potential to influence the electrofishing capture probability of hybrid fish. As such, where a sub-set of captured fish are genetically sampled (i.e. the first 30 caught), there is a risk of biased sampling. To quantify this effect, it would be necessary to screen all fish caught at a number of three-pass electrofishing sites, where both wild and hybrid fish were observed.

Farmed fish of Scottish origin strains

The work presented here is focused on fish of Norwegian origin farmed strains as these are by far the biggest proportion of stocks in aquaculture production in Scotland (~90%). However, strains of Scottish origin have also been used and it is unknown how the marker panel developed and used here would quantify any genetic changes in wild populations as a result of interbreeding with such fish. The genetic character of these fish has not yet been established, and it would be useful in future work to obtain reference samples of these. Testing of the SNP panel could then take place to determine its utility, and if necessary, a bespoke panel could be developed if the present one was not sufficient to detect potential genetic changes arising from these sources.

Effect of genetic status on the population health of wild salmon

Enhancing the understanding of observed patterns of introgression through the various measures outlined above will provide powerful tools to better inform knowledge-based management of the aquaculture industry. Hybridisation and introgression have implications for the fitness and long-term viability of populations (McGinnity et al., 2003; Glover et al., 2017; Castellani et al., 2018). As such, the impacts of the patterns of introgression should also be examined. Integration of NEPS density estimates, with benchmark expected fish densities and levels of genetic change, together with other factors may allow the effects of introgression to start to be quantified. Factors such as the 'health' of impacted populations could be examined and compared to locations with no evidence of genetic change. Furthermore, and importantly, both levels of introgression and population health (in terms of abundance relative to a benchmark) could be examined over time to determine trends. Repeated sampling at a site, or combined temporal trends in regions, could also be examined to determine how levels of introgression influence population trends. Such analyses would move from recognising a risk from introgression to quantifying impact.

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Glossary

Introgression

Introgression is the movement of genetic material of farmed origin into wild salmon populations (Ryman et al., 1995; Karlsson et al., 2016; Glover et al., 2018; Glover et al., 2020). In order to estimate levels of introgression, the proportion of genetic material in each sampled population derived from non-native farmed fish of Norwegian origin is determined (Karlsson et al., 2014; Diserud et al., 2017; Diserud et al., 2020)

Hybridisation

Sexual reproduction between divergent populations (Fraser et al., 2010). In this case hybridisation is intra-specific between two Atlantic salmon from divergent origins, wild and farmed.

Hybrids

The offspring arising from sexual reproduction between farmed and wild salmon and any subsequent generations where the fish have some component of the genome from each original parental type (Fraser et al., 2008; Sylvester et al., 2019). The first generation cross between a farmed and wild salmon are referred to as the F1 hybrid. Subsequent matings between F1 fish and one of the parental types are referred to as backcrosses. If this mating is between the F1 fish and a farmed fish it is referred to as a first-generation backcross to farmed salmon (BC1F) and if the mating is between the F1 fish and a farmed fish it is referred to as a first-generation backcross to wild salmon (BC1W).

Conspecifics

All organisms belonging to the same species.

Genetic integrity

The natural wild genetic characteristics of a population (Bourret et al., 2011).

Fitness

The survival and reproductive success of an individual (McGinnity et al., 2004; Sylvester et al., 2019).

Single Nucleotide Polymorphism (SNP)

Single nucleotide polymorphisms (SNPs) are variations in a single genetic base that are caused by point mutations at a given nucleotide position in the genome. This gives rise to different variants containing alternative nucleotide bases at each SNP position (Moen et al., 2008; Jin et al., 2016). Differences in the proportions of these variants across multiple SNP

markers in the different source populations types (farm/wild) allow differentiation to be made between them (Karlsson et al., 2011).

References

Araki, H., Cooper, B., and Blouin, M. S. 2007. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science, 318: 100-103.

Araki, H., and Schmid, C. 2010. Is hatchery stocking a help or harm?: Evidence, limitations and future directions in ecological and genetic surveys. Aquaculture, 308, Supplement 1: S2-S11.

Besnier, F., Glover, K. A., Lien, S., Kent, M., Hansen, M. M., Shen, X., and Skaala, O. 2015. Identification of quantitative genetic components of fitness variation in farmed, hybrid and native salmon in the wild. Heredity, 115: 47-55.

Bourret, V., O'Reilly, P. T., Carr, J. W., Berg, P. R., and Bernatchez, L. 2011. Temporal change in genetic integrity suggests loss of local adaptation in a wild Atlantic salmon (*Salmo salar*) population following introgression by farmed escapes. Heredity, 106: 500-510.

Castellani, M., Heino, M., Gilbey, J., Araki, H., Svåsand, T., and Glover, K. A. 2018. Modelling fitness changes in wild Atlantic salmon populations faced by spawning intrusion of domesticated escapees. Evolutionary Applications, 11: 1010-1025.

Cauwelier, E., Verspoor, E., Coulson, M. W., Armstrong, A., Knox, D., Stradmeyer, L., Webster, L. M. I., et al. 2018. Ice sheets and genetics: Insights into the phylogeography of Scottish Atlantic salmon, *Salmo salar* L. Journal of Biogeography, 45: 51-63.

Christie, M. R., Marine, M. L., Fox, S. E., French, R. A., and Blouin, M. S. 2016. A single generation of domestication heritably alters the expression of hundreds of genes. Nature Communications, 7: 10676.

Clifford, S. L., McGinnity, P., and Ferguson, A. 1998a. Genetic change in an Atlantic salmon population resulting from escaped juvenile farm salmon. Journal of Fish Biology, 52: 118-127.

Clifford, S. L., McGinnity, P., and Ferguson, A. 1998b. Genetic changes in Atlantic salmon (*Salmo salar*) populations of Northwest Irish rivers resulting from escapes of adult farm salmon. Canadian Journal of Fisheries and Aquatic Sciences, 55: 358-363.

Côte, J., Roussel, J.-M., Le Cam, S., and Evanno, G. 2014. Outbreeding Depression in Atlantic Salmon Revealed by Hypoxic Stress During Embryonic Development. Evolutionary Biology, 41: 561-571.

Coulson, M. W. 2013. Report on Genetic Tool Development for Distinguishing Farmed vs Wild Fish in Scotland. Rivers and Fisheries Trusts of Scotland: Managing Interactions Aquaculture Project 2011/12. 34 pp. https://www.salmon-trout.org/wp-content/uploads/2017/10/RAFTS-2013-Report-on-Genetic-Tool-Development-for-Distinguishing-Farmed-vs-Wild-Fish-in-Scotland.pdf

Crozier, W. W. 1993. Evidence of genetic interaction between escaped farmed salmon and wild Atlantic salmon (*Salmo salar* L.) in a Northern Irish river. Aquaculture, 113: 19-29.

Crozier, W. W. 2000. Escaped farmed salmon, *Salmo salar* L., in the Glenarm River, Northern Ireland: genetic status of the wild population 7 years on. Fisheries Management and Ecology, 7: 437-446.

Dadswell, M. J., Spares, A. D., Reader, J. M., and Stokesbury, M. J. W. 2010. The North Atlantic subpolar gyre and the marine migration of Atlantic salmon *Salmo salar* : the 'Merry-Go-Round' hypothesis. Journal of Fish Biology, 77: 435-467.

Diserud, O. H., Hindar, K., Karlsson, S., Glover, K., and Skaala, Ø. 2017. Genetic impact of escaped farmed salmon on wild salmon stocks - status 2017. NINA. Trondheim, Norway: Report 1337. 55 pp

Diserud, O. H., Hindar, K., Karlsson, S., Glover, K., and Skaala, Ø. 2020. Genetic impact of escaped farmed Atlantic salmon on wild salmon populations – revised status 2020. Norwegian Institute for Nature Research. Trondheim, Norway: Report 1926. 84 pp. https://brage.bibsys.no/xmlui/handle/11250/2435442

Fleming, I. A., Hindar, K., Mjolnerod, I. B., Jonsson, B., Balstad, T., and Lamberg, A. 2000. Lifetime success and interactions of farm salmon invading a native population. Proceedings of the Royal Society of London. Series B: Biological Sciences, 267: 1517-1523.

Fleming, I. A., Jonsson, B., Gross, M. R., and Lamberg, A. 1996. An experimental study of the reproductive behaviour and success of farmed and wild Atlantic salmon (*Salmo salar*). Journal of Applied Ecology, 33: 893-905.

Fraser, D. J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. Evolutionary Applications, 1: 535-586.

Fraser, D. J., Cook, A. D., Eddington, J. D., Bentzen, P., and Hutchings, J. A. 2008. Mixed evidence for reduced local adaptation in wild salmon resulting from interbreeding with escaped farmed salmon: complexities in hybrid fitness. Evolutionary Applications, 1: 501-512.

Fraser, D. J., Houde, A. L. S., Debes, P. V., O'Reilly, P., Eddington, J. D., and Hutchings, J. A. 2010. Consequences of farmed-wild hybridization across divergent wild populations and multiple traits in salmon. Ecological Applications, 20: 935-953.

Garant, D., Fleming, I. A., Einum, S., and Bernatchez, L. 2003. Alternate male life-history tactics as potential vehicles for speeding introgression of farmed salmon traits into wild populations. Ecology Letters, 6: 541-549.

Gilbey, J., Cauwelier, E., Sampayo, J., Matejusova, I., Allan, C., Graham, J., Stradmeyer, L., et al. 2018a. Identification of the farm of origin of Atlantic salmon smolt escapees in a freshwater Scottish loch using single nucleotide polymorphic markers. ICES Journal of Marine Science, 75: 2182-2192.

Gilbey, J., Coughlan, J., Wennevik, V., Prodohl, P., Stevens, J. R., Garcia-Vazquez, E., Ensing, D., et al. 2018b. A microsatellite baseline for genetic stock identification of European Atlantic salmon (*Salmo salar* L.). ICES Journal of Marine Science, 75: 662-674.

Gilbey, J., Sampayo, J., Coulson, M., Cauwelier, E., Verspoor, E., and Morris, D. Submitted. A SNP marker panel for identifying farmed Norwegian Atlantic salmon and their hybrids in wild Scottish populations. Conservation Genetics Resources.

Gilbey, J., Utne, K. R., Wennevik, V., Beck, A. C., Kausrud, K., Hindar, K., Garcia de Leaniz, C., et al. 2021. The early marine distribution of Atlantic salmon in the North-east Atlantic: a genetically informed stock-specific synthesis. Fish and Fisheries.

Gjedrem, T., and Baranski, M. 2009. Selective Breeding in Aquaculture: An Introduction*.,* Springer, London.

Glover, K. A., Quintela, M., Wennevik, V., Besnier, F., Sørvik, A. G. E., and Skaala, Ø. 2012. Three Decades of Farmed Escapees in the Wild: A Spatio-Temporal Analysis of Atlantic Salmon Population Genetic Structure throughout Norway. PLoS ONE, 7: e43129.

Glover, K. A., Solberg, M. F., Besnier, F., and Skaala, Ø. 2018. Cryptic introgression: evidence that selection and plasticity mask the full phenotypic potential of domesticated Atlantic salmon in the wild. Scientific Reports, 8: 13966.

Glover, K. A., Solberg, M. F., McGinnity, P., Hindar, K., Verspoor, E., Coulson, M. W., Hansen, M. M., et al. 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.

Glover, K. A., Wennevik, V., Hindar, K., Skaala, Ø., Fiske, P., Solberg, M. F., Diserud, O. H., et al. 2020. The future looks like the past: Introgression of domesticated Atlantic salmon escapees in a risk assessment framework. Fish and Fisheries, 21: 1077-1091.

Hansen, L. P. 2006. Migration and survival of farmed Atlantic salmon (*Salmo salar* L.) released from two Norwegian fish farms. ICES Journal of Marine Science, 63: 1211-1217.

Hansen, L. P., and Youngson, A. F. 2010. Dispersal of large farmed Atlantic salmon, *Salmo salar*, from simulated escapes at fish farms in Norway and Scotland. Fisheries Management and Ecology, 17: 28-32.
Harvey, A. C., Skilbrei, O. T., Besnier, F., Solberg, M. F., Sorvik, A. E., and Glover, K. A. 2018. Implications for introgression: has selection for fast growth altered the size threshold for precocious male maturation in domesticated Atlantic salmon? BMC Evol Biol, 18: 188.
Hindar, K., Fleming, I. A., McGinnity, P., and Diserud, O. 2006. Genetic and ecological effects of salmon farming on wild salmon: modelling from experimental results. ICES Journal of Marine Science, 63: 1234-1247.
Hutchings, J. A., and Fraser, D. J. 2008. The nature of fisheries- and farming-induced evolution. Molecular Ecology, 17: 294-313.
Jin, Y., Liu, S., Yuan, Z., Yang, Y., Tan, S., and Liu, Z. 2016. 4 - Catfish genomic studies: progress and perspectives. *In* Genomics in Aquaculture, pp. 73-104. Ed. by S. MacKenzie, and S. Jentoft. Academic Press, San Diego.
Jones, O. R., and Wang, J. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources, 10: 551-555.
Kalinowski, S. T. 2011. The computer program STRUCTURE does not reliably identify the main genetic clusters within species: simulations and implications for human population structure. Heredity, 106: 625-632.
Karlsson, S., Diserud, O. H., Fiske, P., and Hindar, K. 2016. Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations. ICES Journal of Marine Science, 73: 2488-2498.
Karlsson, S., Diserud, O. H., Moen, T., and Hindar, K. 2014. A standardized method for quantifying unidirectional genetic introgression. Ecology and Evolution, 4: 3256-3263.
Karlsson, S., Moen, T., Lien, S., Glover, K., and Hindar, K. 2011. Generic genetic differences between farmed and wild Atlantic salmon identified from a 7K SNP-chip. Molecular Ecology Resources, 11: 247-253.
Lochaber Fisheries Trust. 2017. Lochaber Fisheries Trust Annual Report 2016/17. Fort William, Scotland, U.K. 26 pp
López, M. E., Linderoth, T., Norris, A., Lhorente, J. P., Neira, R., and Yáñez, J. M. 2019. Multiple Selection Signatures in Farmed Atlantic Salmon Adapted to Different Environments Across Hemispheres. Frontiers in Genetics, 10.
Mahlum, S., Vollset, K. W., Barlaup, B. T., Skoglund, H., and Velle, G. 2021. Salmon on the lam: Drivers of escaped farmed fish abundance in rivers. Journal of Applied Ecology, 58: 550-561.
Malcolm, I. A., Millidine, K. J., Jackson, F. L., Glover, R. S., and Fryer, R. J. 2019. Assessing the status of Atlantic salmon (*Salmo salar*) from juvenile electrofishing data collected under the National Electrofishing Programme for Scotland (NEPS). Marine Scotland Science. Scottish Marine and Freshwater Science Vol 10 No 2. 77 pp. https://data.marine.gov.scot/dataset/assessing-status-atlantic-salmon-salmo-salar-juvenile-electrofishing-data-collected-under
Malcolm, I. A., Millidine, K. J., Jackson, F. L., Glover, R. S., and Fryer, R. J. 2020. The National Electrofishing Programme for Scotland (NEPS) 2019. Marine Scotland Science. Scottish Marine and Freshwater Science Vol 11 No 9. 57 pp. https://data.marine.gov.scot/dataset/national-electrofishing-programme-scotland-neps-2019
McGinnity, P., Prodohl, P. A., Ferguson, A., Hynes, R., Maoileidigh, N. O., Baker, N., Cotter, D., et al. 2003. Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270: 2443-2450.
McGinnity, P., Prodohl, P. A., O Maoileidigh, N., Hynes, R., Cotter, D., Baker, N., O'Hea, B., et al. 2004. Differential lifetime success and performance of native and non-native

Atlantic salmon examined under communal natural conditions. Journal of Fish Biology, 65: 173-187.
McGinnity, P., Stone, C., Taggart, J., Cooke, D., Cotter, D., Hynes, R., McCamley, C., et al. 1997. Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. ICES Journal of Marine Science, 54: 998-1008.
Millidine, K. J., Fryer, R. J., Jackson, F. L., Glover, R. S., and Malcolm, I. A. 2019. Site locations for the National Electrofishing Programme for Scotland (NEPS) and west coast SAC rivers (2018). Marine Scotland Science. DOI: 10.7489/12112-2
Moen, T., Hayes, B., Baranski, M., Berg, P. R., Kjoglum, S., Koop, B. F., Davidson, W. S., et al. 2008. A linkage map of the Atlantic salmon (*Salmo salar*) based on EST-derived SNP markers. BMC Genomics, 9: 223.
Munro, L. A. 2020. Scottish Fish Farm Production Survey 2019. Marine Scotland Science. Aberdeen, U.K. 55 pp
Naylor, R., Hindar, K., Fleming, I. A., Glodburg, R., Williams, S., Volpe, J., Whoriskey, F., et al. 2005. Fugitive salmon: assessing the risks of escaped fish from net-pen aquaculture. Bioscience, 55: 427-437.
Ounsley, J. P., Gallego, A., Morris, D. J., and Armstrong, J. D. 2019. Regional variation in directed swimming by Atlantic salmon smolts leaving Scottish waters for their oceanic feeding grounds—a modelling study. ICES Journal of Marine Science, 77: 315-325.
Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of Population Structure Using Multilocus Genotype Data. Genetics, 155: 945-959.
Prodöhl, P. A., Ferguson, A., Bradley, C. R., Ade, R., Roberts, C., Keay, E. J., Costa, A. R., et al. 2019. Impacts of acidification on brown trout *Salmo trutta* populations and the contribution of stocking to population recovery and genetic diversity. Journal of Fish Biology, 95: 719-742.
Reed, T. E., Prodohl, P., Hynes, R., Cross, T., Ferguson, A., and McGinnity, P. 2015. Quantifying heritable variation in fitness-related traits of wild, farmed and hybrid Atlantic salmon families in a wild river environment. Heredity, 115: 173-184.
Ryman, N., Utter, F., and Hindar, K. 1995. Introgression, supportive breeding, and genetic conservation. *In* Population management for survival and recovery, pp. 341-366. Ed. by J. Ballou, M. Gilpin, and T. Foose. Columbia University Press, New York, USA.
Skaala, Ø., Besnier, F., Borgstrøm, R., Barlaup, B., Sørvik, A. G., Normann, E., Ostebo, B. I., et al. 2019. An extensive common-garden study with domesticated and wild Atlantic salmon in the wild reveals impact on smolt production and shifts in fitness traits. Evolutionary Applications, 12: 1001-1016.
Skaala, Ø., Glover, K. A., Barlaup, B. T., Svåsand, T., Besnier, F., Hansen, M. M., and Borgstrøm, R. 2012. Performance of farmed, hybrid, and wild Atlantic salmon (*Salmo salar*) families in a natural river environment. Canadian Journal of Fisheries and Aquatic Sciences, 69: 1994-2006.
Solberg, M. F., Skaala, Ø., Nilsen, F., and Glover, K. A. 2013. Does Domestication Cause Changes in Growth Reaction Norms? A Study of Farmed, Wild and Hybrid Atlantic Salmon Families Exposed to Environmental Stress. PLoS ONE, 8: e54469.
Sylvester, E. V. A., Wringe, B. F., Duffy, S. J., Hamilton, L. C., Fleming, I. A., Castellani, M., Bentzen, P., et al. 2019. Estimating the relative fitness of escaped farmed salmon offspring in the wild and modelling the consequences of invasion for wild populations. Evolutionary Applications, 12: 705-717.
Thodesen, J., Grisdale-Helland, B., Helland, S. J., and Gjerde, B. 1999. Feed intake, growth and feed utilization of offspring from wild and selected Atlantic salmon (*Salmo salar*). Aquaculture, 180: 237-246.
Thorstad, E. B., Fleming, I. A., McGinnity, P., Soto, D., Wennevik, V., and Whoriskey, F. 2008. Incidence and impacts of escaped farmed Atlantic salmon *Salmo salar* in nature. NINA Special Report 36. 110 pp

Wacker, S., Aronsen, T., Karlsson, S., Ugedal, O., Diserud, O. H., Ulvan, E. M., Hindar, K., et al. 2021. Selection against individuals from genetic introgression of escaped farmed salmon in a natural population of Atlantic salmon. Evolutionary Applications, 14.

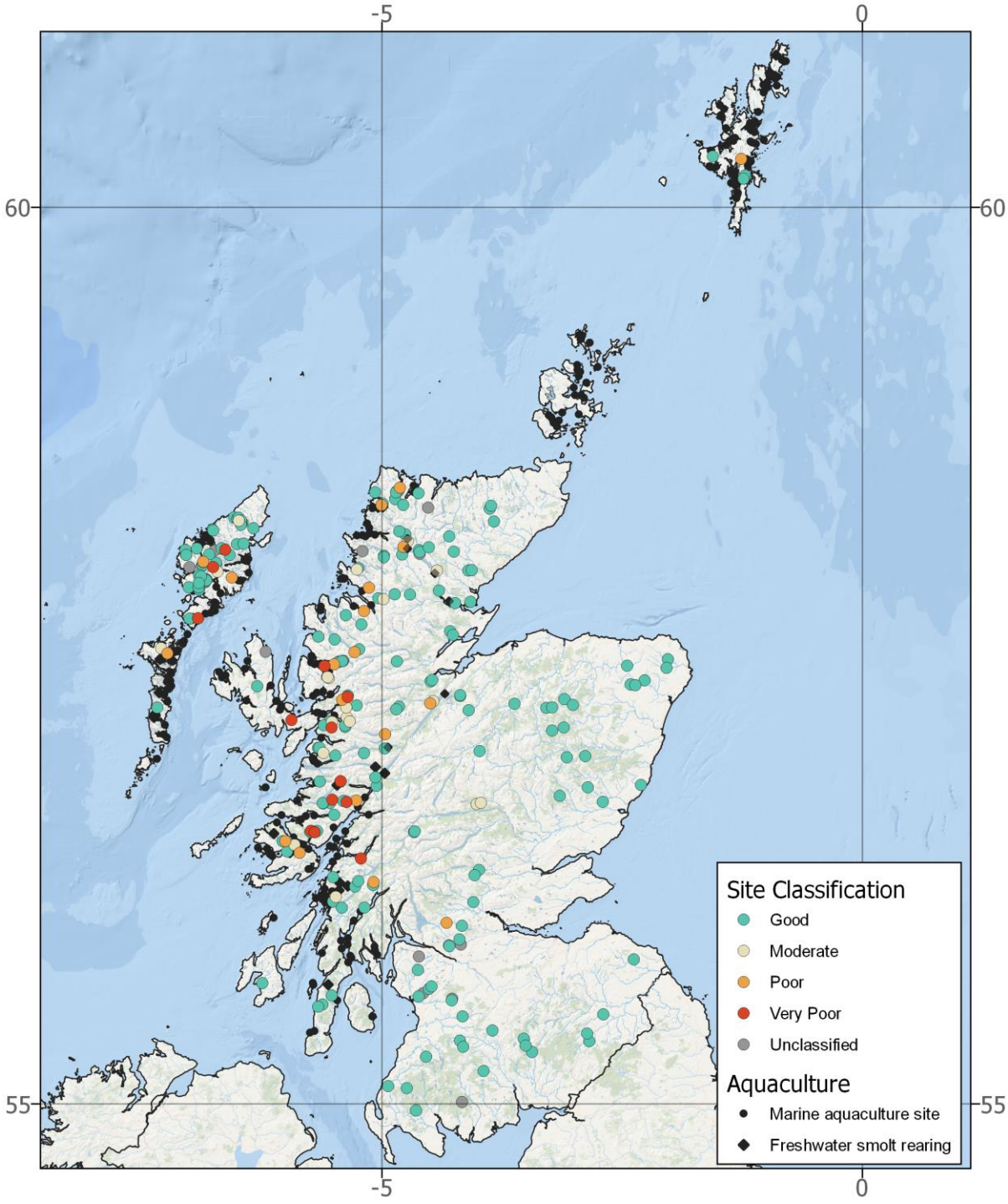
Walker, A. M., Beveridge, C. M., Crozier, W., O Maoileidigh, N., and Milner, N. 2006. Monitoring the incidence of escaped farmed Atlantic salmon, *Salmo salar* L., in rivers and fisheries of the United kingdom and Ireland: current progress and recommendations for future programmes. ICES Journal of Marine Science, 63: 1201-1210.

Walsh, P. S., Metzger, D. A., and Higuchi, R. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques, 10: 506-513.

Warton, D. I., and Hui, F. K. C. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology, 92: 3-10.

Wringe, B. F., Jeffery, N. W., Stanley, R. R. E., Hamilton, L. C., Anderson, E. C., Fleming, I. A., Grant, C., et al. 2018. Extensive hybridization following a large escape of domesticated Atlantic salmon in the Northwest Atlantic. Communications Biology, 1: 108.

Appendix 1: Alternatively coloured map of classifications



Site classification of the genetic status of sampled wild salmon across Scotland in relation to aquaculture production facilities in the marine and freshwater environments.

Appendix 2: Classification details of sites

SiteNo	Site	River	Latitude	Longitude	NEPS Regions	Samples	Sib Families	Classification
1	Annan_0008	Annan	55.38623	-3.522769	Annan	15	0	Green
2	Annan_0020	Annan	55.307975	-3.436598	Annan	15	0	Green
3	Annan_0131	Annan	55.346328	-3.503937	Annan	15	1	Green
4	Argyll_0231	Aray	56.301378	-5.086449	Argyll	5	0	Orange
5	Argyll_0311	Aray	56.284366	-5.091994	Argyll	15	1	Green
6	AFT_AROS_01	Aros	56.535256	-5.989558	Argyll	10	0	Green
7	Aros_SEPA_359101	Aros	56.536664	-6.004467	Argyll	12	0	Orange
8	Aros_SEPA_562025	Aros	56.536219	-6.032674	Argyll	15	0	Green
9	AFT_Avieich_08	Awe	56.275003	-5.278281	Argyll	30	2	Green
10	Argyll_0194	Awe	56.588451	-4.667185	Argyll	7	0	Green
11	Argyll_0224	Awe	56.306504	-5.245857	Argyll	30	1	Green
12	Argyll_0326	Awe	56.592551	-4.65871	Argyll	11	1	Green
13	Argyll_0360	Awe	56.152955	-5.416865	Argyll	3	0	Green
14	Lower_Awe	Awe	56.43677	-5.21816	Argyll	2	0	Red
15	AFT_BA_02	Ba	56.477125	-5.986616	Argyll	29	1	Green
16	AFT_Aros_Barbreck	Barbreck	56.188659	-5.509412	Argyll	15	0	Green
17	AFT_Barbreck_07	Barbreck	56.215589	-5.479751	Argyll	10	0	Yellow
18	Argyll_0185	Barr_Water	55.587886	-5.618338	Argyll	17	1	Green
19	Argyll_0201	Barr_Water	55.575123	-5.663237	Argyll	9	0	Green
20	Argyll_0225	Carradale_Water	55.641716	-5.522178	Argyll	4	0	Green
21	AFT_Euchar_04	Euchar	56.329847	-5.507922	Argyll	30	1	Green
22	AFT_FORSA_03	Forsa	56.51528	-5.90917	Argyll	29	1	Yellow
23	Argyll_0312	Forsa	56.469144	-5.856254	Argyll	3	0	Orange
24	Argyll_0206	Laggan	55.713607	-6.244263	Argyll	13	2	Green
25	AFT_Leacann_11	Leacann_Water	56.153415	-5.181026	Argyll	30	2	Green
26	Ayrshire_0407	Ayr	55.518046	-4.159853	Ayrshire	15	0	Green

SiteNo	Site	River	Latitude	Longitude	NEPS Regions	Samples	Sib Families	Classification
27	Ayrshire_0383	Irvine	55.653751	-4.571683	Ayrshire	1	0	NA
28	Ayrshire_0411	Irvine	55.6354	-4.617833	Ayrshire	4	0	Green
29	Ayrshire_0419	Irvine	55.612418	-4.273527	Ayrshire	9	0	Green
30	Ayrshire_0420	Irvine	55.680323	-4.510384	Ayrshire	5	0	Green
31	Ayrshire_0499	Irvine	55.62201	-4.269095	Ayrshire	1	0	NA
32	Ayrshire_0500	Irvine	55.69417	-4.483646	Ayrshire	2	0	Green
33	Ayrshire_0366	Stinchar	55.093595	-4.740617	Ayrshire	15	0	Green
34	Ayrshire_0402	Stinchar	55.105503	-4.934375	Ayrshire	18	0	Green
35	Ayrshire_0369	Water_of_Girvan	55.280532	-4.539881	Ayrshire	15	1	Green
36	Brora_Helmsdale_0730	Brora	58.056806	-4.10119	Brora/Helmsdale	15	0	Green
37	Brora_Helmsdale_0768	Brora	58.160698	-4.25189	Brora/Helmsdale	15	0	Green
38	Brora_Helmsdale_0854	Brora	58.059181	-4.064784	Brora/Helmsdale	15	0	Green
39	Clyde_1092	Clyde	55.938612	-4.177668	Clyde	1	0	NA
40	Clyde_1128	Clyde	55.869148	-4.614746	Clyde	1	0	NA
41	Clyde_1220	Clyde	55.7909	-4.625484	Clyde	4	0	Green
42	Clyde_1240	Clyde	55.93386	-4.302092	Clyde	16	0	Green
43	Clyde_1245	Clyde	55.968945	-4.192801	Clyde	15	0	Green
44	Clyde2019-02	Clyde	55.929601	-4.298654	Clyde	3	0	Green
45	Clyde_1090	Leven	56.06514	-4.327409	Clyde	9	0	Orange
46	Clyde_1121	Leven	56.048014	-4.166874	Clyde	17	0	Green
47	Conon_1307	Alness	57.720969	-4.288444	Conon	15	0	Green
48	Conon_1308	Alness	57.700488	-4.260131	Conon	15	1	Green
49	Conon_1273	Conon	57.553732	-4.844015	Conon	15	0	Green
50	Dee_190	Dee	57.0144	-3.075326	Dee	13	0	Green
51	Dee_314	Dee	57.020887	-2.883591	Dee	4	1	Green
52	Deveron_1623	Deveron	57.342097	-3.100174	Deveron	15	0	Green
53	Deveron_1640	Deveron	57.529151	-2.447613	Deveron	15	0	Green
54	Deveron_1751	Deveron	57.309715	-3.010909	Deveron	15	0	Green

SiteNo	Site	River	Latitude	Longitude	NEPS Regions	Samples	Sib Families	Classification
55	Don_1809	Don	57.165198	-3.229316	Don	3	0	Green
56	Don_1813	Don	57.182802	-3.10718	Don	14	1	Green
57	Esk_1982	Bervie_Water	56.857436	-2.303712	Esk	15	0	Green
58	Esk_2000	North_Esk	56.843865	-2.857448	Esk	15	0	Green
59	Esk_2111	North_Esk	56.761408	-2.701156	Esk	18	2	Green
60	Esk_2120	South_Esk	56.79556	-3.148771	Esk	15	0	Green
61	Forth_2326	Forth	56.185457	-4.048485	Forth	9	0	Green
62	Galloway_2479	Bladnoch	54.960122	-4.645452	Galloway	26	4	Green
63	Galloway_2353	Dee	55.010816	-4.165948	Galloway	1	0	NA
64	Charlabhaigh_SEPA_562013	Abhainn_Charlabhaigh	58.281601	-6.761663	Hebrides	15	0	Green
65	Langavat_054	Abhainn_Ghriomarstaidh	57.999961	-6.833397	Hebrides	11	0	Green
66	Langavat_055	Abhainn_Ghriomarstaidh	58.075825	-6.756328	Hebrides	3	0	Red
67	Langavat_056	Abhainn_Ghriomarstaidh	58.095772	-6.725317	Hebrides	1	0	NA
68	Langavat_062	Abhainn_Ghriomarstaidh	58.007424	-6.825457	Hebrides	3	0	Green
69	Langavat_063	Abhainn_Ghriomarstaidh	58.098625	-6.726749	Hebrides	10	1	Green
70	Langavat_098	Abhainn_Ghriomarstaidh	58.005998	-6.827468	Hebrides	15	0	Green
71	Langavat_Grimersta	Abhainn_Ghriomarstaidh	58.166459	-6.739865	Hebrides	1	0	NA
72	Langavat_March	Abhainn_Ghriomarstaidh	58.146336	-6.796737	Hebrides	9	0	Green
73	NHarris_109	Abhainn_Ghriomarstaidh	58.002586	-6.828481	Hebrides	4	0	Green
74	OuterHebrides_3608	Abhainn_Ghriomarstaidh	57.992651	-6.83944	Hebrides	1	0	NA
75	OuterHebrides_3742	Abhainn_Mhor_Aglinne_Ruaidh	58.177844	-6.925907	Hebrides	4	0	Green
76	OuterHebrides_3744	Abhainn_Mor_Kintaravay	58.050642	-6.710828	Hebrides	12	0	Yellow
77	OuterHebrides_3615	Abhainn_Tamanabhaigh	58.075273	-7.001328	Hebrides	1	0	NA
78	OuterHebrides_3602	Barvas	58.348259	-6.538225	Hebrides	9	0	Green
79	OuterHebrides_3609	Barvas	58.318906	-6.452017	Hebrides	15	3	Green
80	OuterHebrides_3613	Barvas	58.327546	-6.483726	Hebrides	2	0	Green
81	OuterHebrides_3618	Barvas	58.33463	-6.497861	Hebrides	12	0	Green
82	OuterHebrides_3641	Barvas	58.324535	-6.477771	Hebrides	8	0	Green

SiteNo	Site	River	Latitude	Longitude	NEPS Regions	Samples	Sib Families	Classification
83	OuterHebrides_3645	Barvas	58.339945	-6.505807	Hebrides	18	0	Green
84	OuterHebrides_3650	Barvas	58.313309	-6.462164	Hebrides	15	2	Green
85	OuterHebrides_3653	Barvas	58.346708	-6.492522	Hebrides	5	0	Green
86	OuterHebrides_3737	Barvas	58.33005	-6.489498	Hebrides	15	0	Yellow
87	OuterHebrides_3745	Barvas	58.32893	-6.485543	Hebrides	7	0	Green
88	OuterHebrides_3757	Barvas	58.330254	-6.46521	Hebrides	3	0	Green
89	OuterHebrides_3774	Barvas	58.307429	-6.466207	Hebrides	15	1	Green
90	OuterHebrides_3603	Blackwater_Lewis	58.17476	-6.657649	Hebrides	7	0	Green
91	OuterHebrides_3646	Blackwater_Lewis	58.170994	-6.630208	Hebrides	2	0	Red
92	OuterHebrides_3766	Blackwater_Lewis	58.17948	-6.667803	Hebrides	3	0	Green
93	OuterHebrides_3643	Carloway	58.277068	-6.755145	Hebrides	15	0	Green
94	OuterHebrides_3747	Caslavat	58.142735	-7.037724	Hebrides	15	0	Green
95	Red_River,_SEPA_542179	Caslavat	58.162766	-7.039694	Hebrides	4	0	Green
96	Red_River,_SEPA_542186	Caslavat	58.1697	-7.044764	Hebrides	3	0	Green
97	Red_River,_SEPA_542206	Caslavat	58.142524	-7.03715	Hebrides	10	0	Green
98	OuterHebrides_3607	Creed	58.201552	-6.50873	Hebrides	8	0	Green
99	OuterHebrides_3735	Creed	58.204093	-6.438637	Hebrides	15	1	Green
100	SEPA_2019_01	Eishken	58.018001	-6.561705	Hebrides	15	2	Orange
101	OuterHebrides_3604	Gaireann_System	57.626897	-7.297837	Hebrides	15	2	Yellow
102	OuterHebrides_3617	Gress	58.288804	-6.337108	Hebrides	2	0	Green
103	OuterHebrides_3648	Howmore	57.294963	-7.336755	Hebrides	30	4	Green
104	OHFT_Huamavet_2	Huamavat	57.793503	-6.913571	Hebrides	7	0	Red
105	OuterHebrides_3630	Laxay	58.142189	-6.584107	Hebrides	12	0	Green
106	NHarris_107	Leosavay	57.964293	-7.009865	Hebrides	6	0	Green
107	OHFT_Sgealtair_5	Loch_An_Strumore	57.598021	-7.231494	Hebrides	6	0	Orange
108	NHarris_106	Meavaig	57.952141	-6.901792	Hebrides	2	0	Green
109	NHarris_114	Meavaig	57.964754	-6.907377	Hebrides	4	0	Green
110	SEPA_2019_02	Meavaig	57.964813	-6.907503	Hebrides	15	2	Green

SiteNo	Site	River	Latitude	Longitude	NEPS Regions	Samples	Sib Families	Classification
111	SEPA_2019_03	Meavaig	57.987714	-6.904324	Hebrides	8	0	Green
112	Morsgail_SEPA_562017	Morsgail	58.082555	-6.873167	Hebrides	7	0	Green
113	Morsgail_SEPA_562018	Morsgail	58.086481	-6.865771	Hebrides	8	0	Green
114	Morsgail_SEPA_562019	Morsgail	58.105116	-6.859088	Hebrides	9	0	Orange
115	OuterHebrides_3626	Morsgail	58.101771	-6.859515	Hebrides	7	0	Green
116	NHarris_115	Resort	58.031311	-6.904065	Hebrides	8	0	Green
117	OuterHebrides_3624	Resort	58.025234	-6.882713	Hebrides	4	0	Green
118	OHFT_Steisvat_4	Steisavat	57.791795	-7.00123	Hebrides	24	1	Green
119	Kyle_Sutherland_2563	Carron	57.924494	-4.707646	Kyle of Sutherland	23	1	Green
120	Kyle_Sutherland_2656	Evelix	57.884685	-4.077541	Kyle of Sutherland	15	0	Green
121	KSFT_Shin_1	OykelCassleyShin	58.255392	-4.755386	Kyle of Sutherland	24	5	Green
122	KSFT_Shin_2	OykelCassleyShin	58.217553	-4.729394	Kyle of Sutherland	30	2	Yellow
123	KSFT_Shin_3	OykelCassleyShin	58.186735	-4.775845	Kyle of Sutherland	30	4	Orange
124	KSFT_Shin_4a	OykelCassleyShin	58.148174	-4.606484	Kyle of Sutherland	1	0	NA
125	KSFT_Shin_4b	OykelCassleyShin	58.169744	-4.615036	Kyle of Sutherland	23	1	Green
126	KSFT_Shin_4c	OykelCassleyShin	58.159172	-4.606048	Kyle of Sutherland	7	0	Green
127	KSFT_Shin_5	OykelCassleyShin	58.184794	-4.511837	Kyle of Sutherland	30	1	Green
128	KSFT_Shin_6	OykelCassleyShin	58.056746	-4.42688	Kyle of Sutherland	30	2	Yellow
129	KSFT_Shin_7	OykelCassleyShin	57.945948	-4.406564	Kyle of Sutherland	29	0	Green
130	Kyle_Sutherland_2521	OykelCassleyShin	58.144648	-4.789203	Kyle of Sutherland	15	0	Green
131	Kyle_Sutherland_2523	OykelCassleyShin	57.926297	-4.865868	Kyle of Sutherland	15	0	Green
132	KSFT_Migdale	Springdale_Burn	57.877211	-4.237563	Kyle of Sutherland	12	1	Green
133	Lochaber_059	Ailort	56.876946	-5.646713	Lochaber	3	0	Green
134	Aline_SEPA_202226	Aline	56.594359	-5.673716	Lochaber	4	0	Green
135	Aline_SEPA_232857	Aline	56.588221	-5.745291	Lochaber	6	0	Green
136	Aline_SEPA_562189	Aline	56.587829	-5.700473	Lochaber	15	2	Yellow
137	Lochaber_011	Aline	56.590683	-5.712078	Lochaber	15	0	Green
138	Lochaber_023	Aline	56.595667	-5.738075	Lochaber	3	0	Red

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139	Lochaber_055	Aline	56.587947	-5.700436	Lochaber	13	1	Red
140	Lochaber_134	Carnach	57.024197	-5.484569	Lochaber	14	1	Green
141	Carnoch	Carnoch	56.687	-5.497	Lochaber	3	0	Green
142	Lochaber_058	Gleann_Na_Guiserein	57.067673	-5.664598	Lochaber	7	0	Green
143	Lochaber_138	Gleann_Na_Guiserein	57.068954	-5.649985	Lochaber	9	0	Green
144	Lochaber_142	Inverie	57.038922	-5.60889	Lochaber	11	1	Yellow
145	Lochaber_013	Lochy	56.852148	-5.054716	Lochaber	15	1	Green
146	Lochaber_137	Lochy	56.900911	-5.06683	Lochaber	15	1	Green
147	Lochaber_017	Scaddle	56.76872	-5.265465	Lochaber	9	0	Orange
148	Lochaber_019	Scaddle	56.760507	-5.367978	Lochaber	3	0	Red
149	Lochaber_046	Scaddle	56.76613	-5.401222	Lochaber	2	0	Green
150	Finnan_SEPA_542187	Shiel_Foot	56.876556	-5.431302	Lochaber	15	3	Red
151	Hurich_SEPA_542190	Shiel_Foot	56.773402	-5.516218	Lochaber	3	0	Red
152	Hurich_SEPA_542191	Shiel_Foot	56.754886	-5.539869	Lochaber	11	0	Green
153	Lochaber_160	Shiel_Foot	56.878959	-5.428567	Lochaber	15	1	Red
154	Polloch_SEPA_542192	Shiel_Foot	56.752331	-5.611963	Lochaber	14	1	Green
155	Polloch_SEPA_542193	Shiel_Foot	56.75436	-5.615775	Lochaber	14	1	Green
156	Nairn_Findhorn_Lossie2898	Findhorn	57.279618	-4.096811	Nairn/Findhorn/Lossie	15	0	Green
157	Nairn_Findhorn_Lossie2885	Nairn	57.360291	-4.184812	Nairn/Findhorn/Lossie	15	1	Green
158	Nairn_Findhorn_Lossie2889	Nairn	57.364608	-4.185651	Nairn/Findhorn/Lossie	15	1	Green
159	Beauly_0545	Beauly	57.441003	-4.493349	Ness & Beauly	5	0	Green
160	Beauly_0550	Beauly	57.300626	-4.821581	Ness & Beauly	15	0	Green
161	Beauly_0681	Beauly	57.449633	-4.476301	Ness & Beauly	9	0	Green
162	Beauly_0695	Beauly	57.284565	-4.849096	Ness & Beauly	15	0	Green
163	Ness_3068	Ness	57.064164	-4.968581	Ness & Beauly	3	0	Green
164	Ness_3075	Ness	57.037766	-5.186036	Ness & Beauly	4	0	Green
165	Ness_3079	Ness	57.144753	-4.966207	Ness & Beauly	15	2	Orange
166	Ness_3193	Ness	57.331304	-4.46484	Ness & Beauly	10	0	Yellow

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167	Ness_3213	Ness	57.318462	-4.496269	Ness & Beauly	29	2	Orange
168	Nith_3246	Nith	55.435389	-3.849844	Nith	15	0	Green
169	Nith_3254	Nith	55.375059	-4.187676	Nith	15	0	Green
170	Nith_3255	Nith	55.195424	-3.942508	Nith	18	0	Green
171	Nith_3259	Nith	55.338792	-4.15379	Nith	15	0	Green
172	Northern_3426	Halladale	58.395567	-3.877706	Northern	15	1	Green
173	Northern_3468	Halladale	58.411715	-3.86321	Northern	15	0	Green
174	Northern_3554	Halladale	58.326045	-3.833665	Northern	15	1	Green
175	Northern_3470	Kinloch	58.401043	-4.518603	Northern	1	0	NA
176	Northern_3582	Naver	58.244777	-4.299717	Northern	15	0	Green
177	SEPA_2019_06	Burn_Of_Brouster	60.263964	-1.555957	Shetland (non NEPS)	5	0	Green
178	SEPA_2019_04	Burn_Of_Dale	60.165407	-1.220182	Shetland (non NEPS)	4	0	Green
179	SEPA_2019_05	Burn_Of_Dale	60.14861	-1.23614	Shetland (non NEPS)	3	0	Green
180	SEPA_2019_07	Burn_Of_Sandwater	60.250922	-1.260586	Shetland (non NEPS)	15	0	Orange
181	Spey_001	Spey	57.292893	-3.289375	Spey	15	1	Green
182	Spey_019	Spey	57.316145	-3.620797	Spey	18	0	Green
183	Spey_046	Spey	57.29526	-3.227893	Spey	15	0	Green
184	Spey_139	Spey	57.04953	-3.983069	Spey	15	0	Green
185	Tay_4004	Tay	56.371044	-3.989001	Tay	15	2	Green
186	Tay_4096	Tay	56.340767	-4.03509	Tay	15	0	Green
187	Tay_4114	Tay	56.748299	-4.013857	Tay	15	0	Yellow
188	Tay_4134	Tay	56.755385	-3.970217	Tay	17	0	Yellow
189	Tweed_4148	Tweed	55.371734	-2.841904	Tweed	15	0	Green
190	Tweed_4152	Tweed	55.417193	-2.863929	Tweed	15	0	Green
191	Tweed_4159	Tweed	55.852273	-2.37835	Tweed	15	1	Green
192	Tweed_4160	Tweed	55.530073	-2.695939	Tweed	14	0	Green
193	Ugie_4338	Ugie	57.565199	-2.023504	Ugie	14	1	Green
194	Ugie_4451	Ugie	57.517149	-2.037645	Ugie	15	0	Green

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195	Ugie_4330	Ythan	57.424321	-2.411498	Ugie	10	0	Green
196	Ugie_4335	Ythan	57.447286	-2.266034	Ugie	15	0	Green
197	Ugie_4340	Ythan	57.420366	-2.362471	Ugie	6	0	Green
198	WestSutherland_4502	Abhainn_Aisir_Mhor	58.484741	-5.070577	West Sutherland	6	1	Green
199	WSFT_Thull_1	Allt_Segeir_a_Chadha	58.415278	-5.003622	West Sutherland	30	5	Orange
200	Dionard_Main	Dionard	58.447136	-4.861847	West Sutherland	3	0	Green
201	WestSutherland_4511	Dionard	58.492189	-4.827658	West Sutherland	1	0	NA
202	WestSutherland_4523	Dionard	58.486362	-4.851368	West Sutherland	2	0	Green
203	WestSutherland_4546	Dionard	58.509852	-4.812286	West Sutherland	15	0	Orange
204	WestSutherland_4551	Dionard	58.48399	-4.852977	West Sutherland	2	0	Green
205	WestSutherland_4652	Hope	58.479711	-4.618466	West Sutherland	7	0	Green
206	WestSutherland_4529	Inver	58.137537	-4.978363	West Sutherland	15	2	Green
207	WestSutherland_4541	Inver	58.162813	-5.201736	West Sutherland	1	0	NA
208	WestSutherland_4665	Inver	58.130438	-4.979486	West Sutherland	15	0	Green
209	WestSutherland_4634	Laxford	58.271968	-4.81857	West Sutherland	15	0	Green
210	WestSutherland_4552	Polla	58.415931	-4.778041	West Sutherland	5	0	Green
211	WestSutherland_4505	Polly	58.062217	-5.2603	West Sutherland	15	0	Yellow
212	WestSutherland_4509	Polly	58.070986	-5.245697	West Sutherland	15	0	Green
213	WSFT_Rhiconich	Rhiconich	58.422721	-4.990861	West Sutherland	4	0	Green
214	Balgy_SEPA_542202	Balgy	57.464273	-5.563611	Wester Ross	13	0	Yellow
215	Balgy_SEPA_542203	Balgy	57.459952	-5.553032	Wester Ross	14	0	Yellow
216	WHFT_Balgy_1	Balgy	57.527424	-5.597903	Wester Ross	30	2	Red
217	WHFT_Balgy_2a	Balgy	57.467444	-5.571011	Wester Ross	4	0	Green
218	WHFT_Balgy_2b	Balgy	57.4656	-5.565025	Wester Ross	21	2	Yellow
219	WesterRoss_Skye_4814	Broadford	57.224804	-5.94121	Wester Ross	15	0	Red
220	SEPA_2019_08	Croe	57.240575	-5.365657	Wester Ross	15	0	Yellow
221	SEPA_2019_09	Croe	57.216677	-5.33551	Wester Ross	15	1	Yellow
222	WesterRoss_Skye_4685	Dundonnell	57.833058	-5.186274	Wester Ross	15	0	Orange

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223	WesterRoss_Skye_4682	Elchaig	57.295072	-5.371202	Wester Ross	15	0	Yellow
224	WesterRoss_Skye_4698	Elchaig	57.30697	-5.262702	Wester Ross	13	3	Green
225	WesterRoss_Skye_4683	Ewe	57.674895	-5.492108	Wester Ross	7	0	Green
226	WesterRoss_Skye_4703	Ewe	57.620832	-5.236388	Wester Ross	12	1	Green
227	WesterRoss_Skye_4731	Ewe	57.605179	-5.284578	Wester Ross	15	0	Orange
228	WesterRoss_Skye_4693	Glenmore	57.218157	-5.543519	Wester Ross	15	2	Green
229	WesterRoss_Skye_4725	Glenmore	57.182404	-5.52342	Wester Ross	11	1	Red
230	WesterRoss_Skye_4825	Glenmore	57.213809	-5.51785	Wester Ross	15	0	Yellow
231	WesterRoss_Skye_4829	Glenmore	57.194679	-5.60395	Wester Ross	15	0	Green
232	WesterRoss_Skye_4681	Gruinard	57.758563	-5.213357	Wester Ross	15	1	Green
233	WesterRoss_Skye_4735	Gruinard	57.809921	-5.375519	Wester Ross	14	1	Green
234	WesterRoss_Skye_4721	Kanaird	57.962399	-5.134098	Wester Ross	9	0	Orange
235	Pmussel_WesterRoss_010	Kerry	57.691139	-5.661763	Wester Ross	15	1	Green
236	Ling_SEPA_562009	Ling	57.356061	-5.362244	Wester Ross	5	0	Orange
237	Ling_SEPA_562010	Ling	57.33835	-5.414004	Wester Ross	7	0	Orange
238	Ling_SEPA_562011	Ling	57.353466	-5.355754	Wester Ross	14	2	Red
239	Ling_SEPA_562012	Ling	57.362569	-5.354157	Wester Ross	10	0	Green
240	WesterRoss_Skye_4686	Shiel_Bridge	57.185914	-5.381572	Wester Ross	15	0	Green
241	WesterRoss_Skye_4730	Shiel_Bridge	57.193541	-5.384754	Wester Ross	15	0	Green
242	Upper_Snyzart	Snizort	57.414159	-6.296822	Wester Ross	2	0	Green
243	WesterRoss_Skye_4708	Stenscholl	57.606778	-6.210342	Wester Ross	1	0	NA
244	Torridon_SEPA_542195	Torridon	57.555589	-5.413557	Wester Ross	11	1	Green
245	Torridon_SEPA_542196	Torridon	57.556602	-5.402012	Wester Ross	1	0	NA
246	Torridon_SEPA_542199	Torridon	57.552176	-5.434307	Wester Ross	14	1	Green
247	Torridon_SEPA_542200	Torridon	57.550571	-5.454255	Wester Ross	8	0	Green
248	Torridon_SEPA_542201	Torridon	57.537169	-5.498535	Wester Ross	5	0	Orange
249	WesterRoss_Skye_4722	Torridon	57.547533	-5.462428	Wester Ross	15	0	Green
250	WHFT_Torridon	Torridon	57.555214	-5.413472	Wester Ross	30	4	Green

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251	WesterRoss_Skye_4689	Ullapool	57.898687	-4.984507	Wester Ross	13	1	Yellow
252	WesterRoss_Skye_4729	Ullapool	57.90457	-5.036572	Wester Ross	8	0	Green

Sib families refers to the number of full-sib families present. If no full-sibs were found the value will be 0.

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