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# Has stocking contributed to an increase in the rod catch of anadromous trout (*Salmo trutta* L.) in the Shetland Islands, UK?

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**Abstract**

The stocking of hatchery-origin fish into rivers and lakes has long been used in fisheries management to try to enhance catches, especially for trout and salmon species. Frequently, however, the long-term impacts of stocking programmes have not been evaluated. In this study, the authors investigate the contribution of a stocking programme undertaken to support the rod catch of sea trout in the Shetland Islands, UK. Once a highly productive recreational fishery, Shetland sea trout catches crashed in the mid-1990s. Around the time that stocking began, increases in rod catches were also reported, with advocates of the stocking highlighting the apparent success of the programme. Using a suite of genetic markers (microsatellites), this study explores the contribution of the stocking programme to the Shetland sea trout population. The authors found that the domesticated broodstock and wild spawned brown trout from seven streams were genetically distinct. Despite extensive stocking, wild spawned brown trout dominated, even in those streams with a long history of supplementation. The majority of sea trout caught and analysed were of wild origin – only a single individual was of pure stocked origin, with a small number of fish being of wild × stocked origins. This study suggests that stocking with a domesticated strain of brown trout has made only a very limited contribution to the Shetland Islands rod catch, and that the revival of sea trout numbers appears to be driven almost exclusively by recovery of trout spawned in the wild.

**KEYWORDS**
anadromous trout, angling, microsatellite, supplementation

## 1 | INTRODUCTION

Stocking of trout and salmon is generally an attempt to fix a problem, either real or perceived, and is usually undertaken when salmonid stocks have been degraded because of processes such as habitat change and overexploitation, or to enhance catches for commercial or recreational fisheries (Aprahamian *et al.*, 2003). The practice of stocking in salmonid fisheries is now generally recognized as having detrimental effects on the wild populations into which fish are stocked (Araki &

Schmid, 2010; Young, 2013). Effects can include the introgression of "domestic" alleles leading to the loss of local adaptations and competition between wild and hatchery-origin fish for resources such as food and spawning gravels (Brenner *et al.*, 2012; Ferguson, 2007). Longer-term impacts, though, can be variable – sometimes negligible – depending on a number of factors, including the intensity and duration of the original stocking programme and the relative genetic similarity/dissimilarity between the wild recipient population and the exogenous fish (Finnegan & Stevens, 2008; Glover *et al.* 2017).

Long-term maintenance of fish within hatcheries can lead to the effective domestication of broodstocks. Hatchery stocks can quickly become adapted to living in captivity which, in turn, can lead to lower reproductive success of hatchery-bred individuals when released into the wild (Araki *et al.*, 2008; Thériault *et al.*, 2011). Differences in important traits such as predator response, territorial behaviour and general physiology have also been found between wild and domesticated fish stocks (*e.g.*, Lorenzen *et al.*, 2012; Schwinn *et al.*, 2017; Vandersteen *et al.*, 2012). In addition, hatchery and wild stocks are often strongly genetically divergent (Guillerault *et al.*, 2018; Hansen & Mensberg, 2009; Weigel *et al.*, 2019). Indeed, even stocking programmes using supportive-breeding – whereby wild adult fish taken from the river to be stocked are used as broodstock – have been shown to lead to dramatic changes in levels of genetic diversity and structuring if insufficient numbers of wild fish are used to create the broodstock (*e.g.*, Griffiths *et al.*, 2009; Selly *et al.*, 2014). Despite this, supportive-breeding is still often cited as being less detrimental to the genetic diversity of a wild population than exogenous stocking (Solomon *et al.*, 2003). In addition, while in some instances hatchery releases can lead to significantly increased commercial catches, e.g., Alaskan pink salmon (*Oncorhynchus gorbuscha*) (Ruggerone *et al.*, 2010), often releases result in little or no change in rod-catch (Coulson *et al.*, 2013; Young, 2013), while in others the numbers of released fish can result in reduced productivity of wild stocks through negative interactions between wild and hatchery-derived fish (Amoroso *et al.*, 2017).

Nonetheless, despite the generally negative effects of hatchery supplementation on wild populations, stocking programmes are still undertaken in an effort to restore degraded fisheries. One such case concerns sea trout stocks in the Shetland Islands, Scotland, UK. During the 1990s, sea trout rod catches crashed from an average of over 1000 fish per year in the mid to late 1980s to just 40 fish in 1998 (Supporting Information Figure S1) (Scottish Government, 2014). In early 2002, the Shetland Anglers Association (SAA) began stocking lochs and streams in many areas of Shetland with fry derived from domesticated broodstock that had previously been used by a commercial fishery raising smolts for sale to Shetland fish farms. The SAA programme used the same broodstock from its inception, with fish reported to have come originally from the Howietoun trout farm near Stirling, Scotland (Shetland Anglers Association, pers. comm.). The Howietoun trout farm was established from Loch Leven broodstock in 1881 (Maitland, 1887) and has been domesticated since that time (almost 50 generations). Although originally colonised by anadromous trout, Loch Leven fish now constitute an adfluvial-lacustrine migratory stock and sea trout have been absent from the loch since the latter part of the 19th century. The lack of availability of an archive of broodstock material for genetic characterisation is a serious limitation on the precision of any study attempting to assess the impact and contribution of a hatchery release programme.

Concomitant with the stocking programme on Shetland, there has been a steady increase in the rod catch of sea trout to levels not seen since the 1980s (Marine Scotland, 2019). At the time sampling was undertaken for this research, it was the belief of the SAA that a high percentage of trout in stocked waters and the majority of sea trout were of stocked origin (Shetland Anglers Association, pers. comm.).

In this study, using microsatellite markers, the authors investigate three questions related to the SAA stocking programme: (a) From a genetic perspective, are there two groups of trout in the streams and coastal waters of the Shetland Isles – one corresponding to wild-spawned fish and the other to stocked fish? (b) If this is the case, what is the dominant group present in burns that have been supplemented with stocked fish? (c) What is the origin of sea trout caught in the marine environment and in fresh water? Resident trout samples were collected from seven catchments on the east and west side of the Mainland of Shetland and the northern island of Yell (Figure 1). Five of these catchments were stocked regularly between 2006 and 2015 (Supporting Information Table S1). Samples of sea trout were obtained from both coastal and fresh waters.

## 2 | MATERIALS AND METHODS

Fieldwork and electrofishing were carried under permission from Marine Scotland. Live fish were anaesthetised and released immediately after recovery. The majority of tissue samples taken were minimally invasive fin clips. Details of sample location for wild-caught trout and Shetland Angling Association broodstock are given in Table 1 and Figure 1.

Note, due to the SAA having been releasing juvenile trout of hatchery origin into the burns and lochs of Shetland for 13 years prior to the current study, it seems sensible to assume that some stocked fish will have interbred successfully with native trout and that alleles from trout of hatchery origin may have introgressed into the wild resident population. Consequently, the genetic profiles of fish referred to as wild in this paper may include a variable and unquantified genetic component of hatchery-origin. Accordingly, all reference to wild Shetland trout should be taken as meaning putatively wild. Unfortunately, tissues samples of native Shetland trout sampled from the wild before the SAA stocking programme commenced were not available for genetic analysis.

Juvenile trout samples were collected from seven catchments in 2009 and 2015 (Table 1). Adult broodstock fish were sampled in 2015 only; the SAA used eggs/milt from 100 adult broodstock fish per year (50 of each sex) of which the authors sampled 50 (25 of each sex). Fish were sampled from the wild by backpack electrofishing and anaesthetised using MS-222 (Sigma-Aldrich, St. Louis, USA) prior to removal of an adipose fin clip using sharp scissors. Fin clips were transferred immediately into tubes containing absolute ethanol. Additional trout samples (3 × juvenile baseline samples and five sea trout), in the form of brain tissue stored in RNAlater (Sigma-Aldrich) that had been collected as part of an investigation into an outbreak of infectious salmon anaemia virus on a Shetland Atlantic salmon (*Salmo salar* L.) farm (Murray *et al.*, 2010), were obtained from three streams on the west side of Mainland in the near vicinity of the salmon farm by
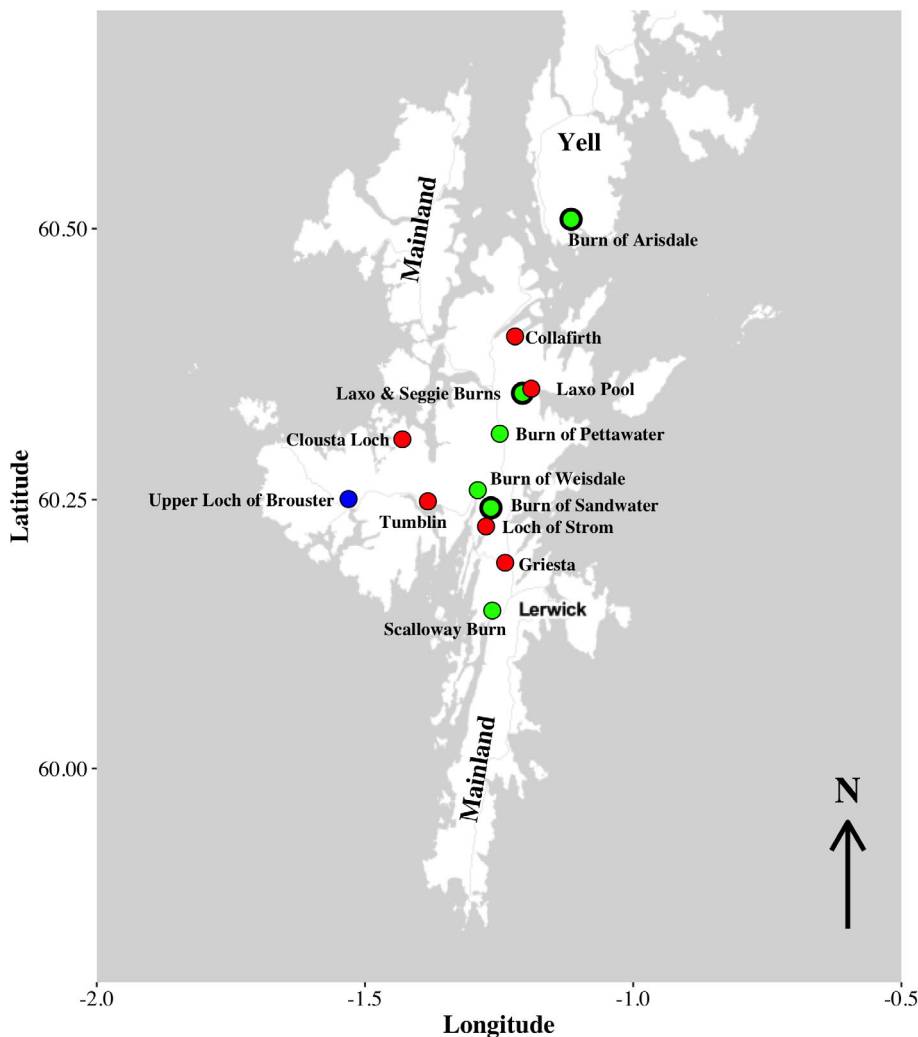
**FIGURE 1** Map showing the location of sample sites of resident trout and anadromous trout in Mainland and Yell, Shetland Islands. Blue circle – location of the Shetland Angling Association broodstock cages in Upper Loch of Brouster; red circles – sea trout sampling sites; green circles – resident trout sampling sites; bold outlined green circles – sites where both resident and sea trout were collected. For clarity, only a single symbol is shown for the Laxo and Seggie Burn sites, which were close together

Marine Scotland (Table 1, Figure 1). Scale samples were taken from sea trout caught on rod and line from multiple freshwater and coastal locations around the Shetland Isles during 2015–2017 (Figure 1; Supporting Information Table S3). An additional 18 sea trout were caught from three catchments (Burn of Sandwater: $n = 6$; Laxo Burn: $n = 3$; Burn of Arisdale (Yell): $n = 9$) in fresh water, whereas electrofishing for juvenile trout and fin clips were taken as described earlier. All fish caught in coastal/estuarine waters were classified as sea trout; fish caught in fresh water were classified on the basis of their appearance (primarily colouration, but also shape and size). Genomic DNA was extracted using the HotSHOT method (Truett et al., 2000).

Samples were screened for variation with 21 nuclear microsatellite primer sets (Supporting Information Table S2). Multiplex PCRs and genotyping were performed as described in Paris et al. (2015). Five loci (Ssa85, BG935488, CA060208, CA060177 and sasaTAP2A) show non-overlapping size ranges between trout and Atlantic salmon and are therefore useful for the identification of salmon and trout × salmon hybrids (King et al., 2016).

The presence of large allele dropout, stuttering and null alleles were determined for each locus using Micro-Checker v2.2 (Van Oosterhout et al., 2004). Linkage disequilibrium (LD) between all pairs of loci within each population was tested for using the R package

genepop (Rousset, 2008). Significance was estimated using a Markov-chain method (10,000 de-memorisations, 100 batches and 5000 iterations). False discovery rate (FDR, Benjamini & Hochberg, 1995) was used to correct significance levels for multiple comparisons.

Brown trout populations can sometimes contain large numbers of closely related individuals (Goodwin et al., 2016; Hansen et al., 1997). False inference of population structure can be obtained when full-sib families are retained within data sets (Andersen & Dunham, 2008; Rodríguez-Ramilo & Wang, 2012). COLONY v 2.0 (Jones & Wang, 2010), which implements a maximum-likelihood method to assign sibship and parentage to individuals based on their multilocus genotype, was used to determine if any of the trout collected from the seven burns were members of full-sib families, and if they had a male or female parent present in the SAA broodstock sample. Conditions were: high precision medium length run, assuming both male and female polygamy without inbreeding, a 1% error rate for both scoring and allelic dropout error rates, and with a 0.25 probability that a father or mother is included in the candidate parental fish. Individuals were considered members of a full-sib family if the probability of exclusion as full-sib families was >0.9. The authors used the Waples and Andreson (2017) Yank-2 method to trim full sibs from the data set – both members of families with two individuals were retained but

**TABLE 1** Details of sampling sites for resident brown trout, Shetland Anglers Association (SAA) broodstock and sea trout[a]

| Code | Year of collection | Catchment[b] | Site | Coordinates (WGS84) | $n_1$ | $n_2$ | Full sib families | Family size |
|------|-------------------|-------------|------|--------------------|------|------|-------------------|-------------|
| SAND | 2009[c] & 2015 | Burn of Sandwater (west Mainland) | Stromfirth | 60.242, −1.265 | 7 | 7 | – | – |
| PETT | 2015 | Burn of Pettawater (west Mainland) | Pettawater outflow | 60.311, −1.249 | 38 | 23 | 2 | 2, 17 |
| SEGG | 2015 | Seggie Burn (east Mainland) | Upstream of B9071 | 60.354, −1.204 | 49 | 45 | 5 | 2, 2, 2, 3, 5 |
| LAXO | 2015 | Laxo Burn (east Mainland) | 250 m upstream of confluence of Laxo Burn and Seggie Burn | 60.347, −1.208 | 45 | 39 | 8 | 2, 2, 2, 3, 3, 3, 4 |
| ARIS | 2015 | Burn of Arisdale (Yell) | 300 m upstream of B9081 bridge | 60.515, −1.120 | 51 | 49 | 7 | 2, 2, 2, 2, 2, 3, 3 |
| SCAL | 2009[c] | Scalloway Burn (west Mainland) | B9074 bridge to Loch of Asta outflow | 60.129, −1.260 | 52 | 48 | 1 | 6 |
| WEIS | 2009[c] | Burn of Weisdale (west Mainland) | Stenswall | 60.257, −1.290 | 18 | 18 | – | – |
| SAA BS | 2015 | SAA broodstock | Upper Loch of Brouster | 60.250, −1.529 | 50 | 46 | 3 | 2, 2, 3 |
| Sea trout[a] | 2009[c] & 2015–17 | Various locations[a] | – | – | 55 | 55 | 2 | 2, 2 |

$n_1$: sample size.

$n_2$: sample size after removal of full-sibs and salmon × trout hybrids (leaving two per full sib group, Waples & Andreson, 2017).

[a]See Supporting Information Table S3 for full details.

[b]The 2015 baseline samples were collected (28–30 September) from water bodies on Mainland of Shetland, except ARIS, which was collected on Yell.

[c]The 2009 baseline samples were collected by Marine Scotland, 23–25 March 2009.

if a family had three or more individuals, all but two random members were removed.

The wild *vs.* domesticated origins of fish were inferred using multiple methods. A model-based clustering method (STRUCTURE v2.3.4, Pritchard *et al.*, 2000) was used to assign individuals to genetic groups. The programme uses a Bayesian-based Markov Chain Monte Carlo (MCMC) approach to jointly define $K$, the number of possible partitions of the data set and the proportion of an individual's genome ($q$) that is derived from each of the $K$ populations. Ten independent runs of 250,000 iterations following a burn-in of 100,000 iterations were performed using the admixture model with correlated allele frequencies and not using the population of origin information on $K$ ranging from 1 to 10. The optimum $K$ for the data set was determined using the Evanno *et al.* (2005) $\Delta K$ method as implemented in POPHELPER v1.0.6 (Francis, 2017).

The posterior probability of individuals being assigned to each of six distinct classes, namely pure broodstock, pure wild, $F_1$, $F_2$, backcross to broodstock and backcross to wild, was computed using a Bayesian model–based method, as implemented in the programme NewHybrids v1.1 (Andersen & Thompson, 2002). Results were based on 100,000 sweeps of the Markov chain, following a 30,000-sweep burn-in period using uniform priors for both mixing proportions and allele frequencies. The programme was run three times using different random number seeds. As runs were highly concordant, final assignments were based on a single representative run. Results were visualised using POPHELPER v1.0.6 (Francis, 2017).

A discriminant analysis of principal components (DAPC; Jombart *et al.*, 2010) analysis was undertaken using the *adegenet* (Jombart, 2008) package for R (R Core Team, 2018). Identification of clusters of

individuals ($K$) was achieved *de novo* using the find.clusters() function, retaining 200 principal coordinates (PCs), and the DAPC analysis was performed on the number of clusters with the lowest Bayesian information criterion (BIC) value, initially retaining 150 PCs and 4 discriminant functions (DFs). The optimum number of principal components to be retained in the analysis was determined using the optim.a.score() function. Results were visualised as both scatter and bar plots.

To assess the power of this study's microsatellite panel to distinguish wild, broodstock and potential hybrid individuals, the authors conducted a simulation study. Using HybridLab (Nielsen *et al.*, 2006), the authors of this study simulated 100 genotypes for each of six genotype classes (pure broodstock, pure wild, $F_1$, $F_2$, backcross to broodstock and backcross to wild). They used the broodstock and Burn of Arisdale genotypes as reference for each "pure" group. Burn of Arisdale was chosen as, apart from the stocking of some smolts in 2015, this site had not been stocked by the SAA. Data for the 600 simulated genotypes were analysed using STRUCTURE ($K = 2$ only) and NewHybrids, as described earlier. For NewHybrids, assignment was considered correct if the true genotype class was the one with the highest posterior probability. Results for both STRUCTURE and NewHybrids were visualised using POPHELPER v1.0.6 (Francis, 2017).

## 3 | RESULTS

A total of 368 fish [263 resident fish, 50 SAA adult broodstock (25 males and 25 females) and 55 sea trout] were analysed. A single Atlantic salmon fry was collected from Seggie Burn and a

single salmon × trout hybrid was collected from Laxo Burn. A further three samples from the SAA broodstock failed to amplify at several loci. These samples were removed from the data set.

The 21 primer sets amplified a total of 22 loci. The primers for One102 amplified two loci with non-overlapping size ranges (designated One102a and One102b – King et al., 2016). Three loci were removed from the data set. Locus CA054565 was almost completely monomorphic for a 111 base-pair (bp) allele. Only two other alleles were found for this locus, both as heterozygotes with the 111 bp allele: a 103 bp allele in SCAL (found in only three individuals) and a 115 bp allele in LAXO (found in only four individuals). Loci CA053292 and SsaD157 were removed due to the presence of null alleles in five and four populations, respectively. In addition, these two loci showed significant deviation from HWE, after FDR correction, in five populations each. For the remaining loci, significant deviations from HWE were found for seven tests comprising four loci and five populations. Tests for LD found that only 2 of 1890 tests were significant after FDR correction. As none of these significant results were consistent across loci or populations, all remaining loci and populations were retained for further analysis. A total of 293 alleles were found for the remaining 19 loci [average 15.4 alleles per locus, 2 (One102a) – 35 (Ssa407UOS) alleles per locus].

Results from running COLONY indicated that the number of full-sib families per sample site ranged from zero to eight (Table 1). The number of individuals per family was generally less than four. Nonetheless, a single family found in the Burn of Pettawater (PETT) contained 17 members (Table 1). Three full-sib families were identified in the broodstock. Paternity was assigned to seven wild-sampled fish, with six broodstock males acting as paternal parents (Table 2). Maternity was assigned to seven wild-sampled fish, including a single sea trout, with six broodstock females acting as maternal parents (Table 2). For three fish, all collected from the PETT, it was possible to determine both male and female broodstock parents (Table 2). After removal of full-sibs, 330 genotypes (46 broodstock, 229 fish from

**TABLE 2** Details of broodstock males and females assigned as parents to resident brown trout and sea trout sampled from Shetland burns

| Male parent | Female parent | Offspring |
| --- | --- | --- |
| SAABS M11 | SAABS F31 | SHET2.03 |
| SAABS M23 | SAABS F38 | SHET2.04 |
| – | SAABS F26 | SHET2.07 |
| SAABS M11 | SAABS F39 | SHET2.08 |
| SAABS M12 | – | SHET2.10 |
| – | SAABS F33 | SHET2.18 |
| SAABS M18 | – | SHET2.22 |
| – | SAABS F38 | SHET4.10 |
| SAABS M05 | – | SHET4.16 |
| SAABS M06 | – | SHET6.40 |
| – | SAABS F42 | SHETSC21[a] |

[a]Sea trout.

burns and 55 sea trout) were retained for subsequent analyses (Table 1).

Results of the STRUCTURE analyses gave $K = 2$ as the optimum partition of the data ($\Delta K = 667.4$), with an additional peak at $K = 5$ ($\Delta K = 228.3$) (Supporting Information Figure S2). For $K = 2$, one group corresponded to the SAA broodstock and the other to putatively wild fish (Figure 2a). Four of the broodstock showed a mix of wild and broodstock ancestry. The majority of fish sampled from the seven burns belonged to the wild group. The PETT had the highest proportion of stocked fish (Figure 2a). The large full-sib family identified at this site was of wild origin. Several fish in the remaining six burns showed varying degrees of mixed ancestry. Burns that had been stocked tended to show a higher proportion of stocked ancestry (i.e., PETT, LAXO and WEIS, Figure 2a). The majority of sea trout were wild fish (Figure 2b). Only a single sea trout was of stocked origin (from Weisdale Pool), with a small number being of wild × stocked origins (Figure 2b).

The results for $K = 5$ showed geographically based genetic structuring, distinguishing wild fish sampled from west Mainland burns from those on east Mainland and Yell (Supporting Information Figure S3). There was further structuring within each of these groups with Scalloway Burn (SCAL) being distinct from the other burns on west Mainland and the Burn of Arisdale (Yell) being distinct from Laxo and Seggie Burns (Supporting Information Figure S3). This partition of the data showed that the sea trout caught in marine waters might be mainly foraging close to their natal rivers. For instance, the 11 sea trout caught in the Loch of Strom belonged to the same genetic group as fish sampled from the Burn of Sandwater, the main freshwater source flowing into the Loch of Strom (Supporting Information Figure S3).

The results of the NewHybrids analyses were highly concordant with the STRUCTURE $K = 2$ analysis, showing that the majority of fish sampled from burns were of wild ancestry with only small numbers of pure domestic fish. Fish that were shown to be admixed in the STRUCTURE analysis were most likely advanced generation hybrids ($F_2$s and backcross to wild) in the NewHybrids results (Supporting Information Figure S4a). There was no evidence of any backcrossing to the domestic broodstock. This pattern is also seen in the sea trout individuals (Supporting Information Figure S4b).

Both STRUCTURE $K = 5$ and NewHybrids show a distinction between sea trout caught on west and east of Shetland. On east Mainland the majority of sea trout were pure wild individuals, whereas several of the sea trout caught on west Mainland show varying degrees of admixture between domestic and wild stocks (Figures 2b and Supporting Information Figure S4b).

Results of the DAPC analysis were in broad agreement with the STRUCTURE $K = 5$ analysis. The first two axes explained 43.53% and 22.59% of the variation, respectively (Figure 3). The optimum number of clusters was $K = 5$ (BIC = 666.72, Supporting Information Figure S5) and the optimal number of PCs retained for analysis was 24. One cluster contained fish of domesticated origins, whereas wild-caught individuals were divided between four clusters: two west Mainland clusters (one containing predominantly SCAL individuals,
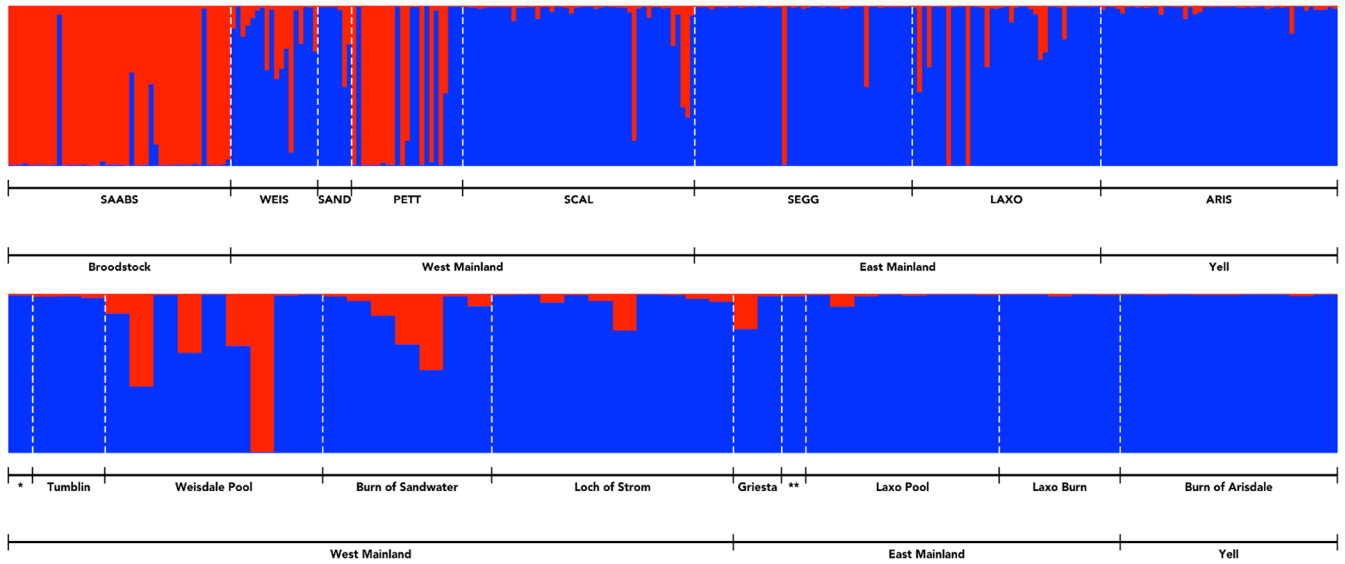
**FIGURE 2** Results of STRUCTURE ($k = 2$, $\Delta K = 355.6$) analysis of (a) non-anadromous trout [263 resident fish, comprising 50 SAA adult broodstock (25 male and 25 female), and fish sampled from WEIS, SAND, PETT, SCAL, SEGG, LAXO, ARIS], and (b) sea trout ($N = 55$) collected from sites across the Shetland Islands (Table 1). Red denotes the domestic genetic group and blue the wild genetic group; sample codes are as given in Table 1. For clarity, sea trout individuals from Clousta Loch and Collafirth are labelled * and **, respectively
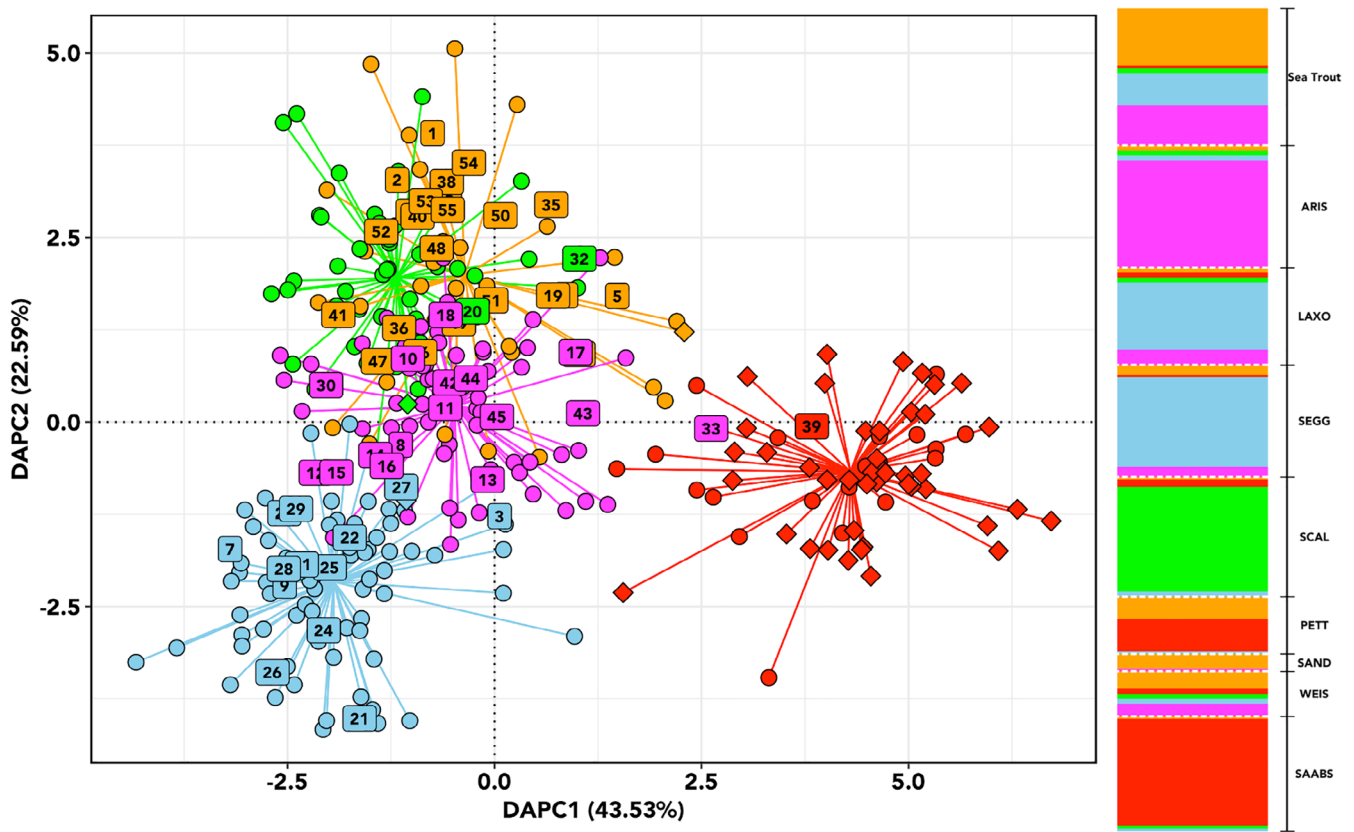


**FIGURE 3** Discriminant analysis of principal components (DAPC) scatter plot (DAPC1 vs. DAPC2) of microsatellite-based genetic profiles for Shetland resident trout and sea trout collections. Each symbol represents the genotype of an individual fish: Shetland Angling Association broodstock = diamonds; fish sampled from burns = circles. Cluster colours: red – domesticated origin; orange – SAND, PETT and WEIS (Sandwater/Pettawater/Weisdale, west Mainland); green – SCAL (Scalloway, west Mainland); purple – ARIS (Arisdale, Yell); sky blue – SEGG and LAXO (Seggie/Laxo, east Mainland). Sea trout are shown as numbered tiles with numbers corresponding to those given in Supporting Information Table S3. The bar plot (right-hand side) shows assignment of individuals from the five clusters identified in the scatter plot to population of origin

SCAL, and a second containing mostly west Mainland fish from SAND, PETT and WEIS), a cluster of Laxo Burn/Seggie Burn fish and a cluster of Burn of Arisdale individuals (Figure 3, Supporting Information Table S4). All sea trout, except one, group within one of the four wild clusters (Figure 3).

Analysis of simulated genotypes showed that both STRUCTURE and NewHybrids were able to reliably distinguish the broodstock, wild and hybrid ($F_1$, $F_2$ and backcrosses combined) genotypes (Supporting Information Figures S6 and S7). Nonetheless, hybrid genotypes were more difficult to assign correctly to specific hybrid classes. STRUC-TURE was not able to consistently distinguish the separate hybrid classes, especially $F_1$ and $F_2$ genotypes (Supporting Information Figure S6). NewHybrids was able to correctly assign the majority of $F_1$ genotypes (Supporting Information Table S5, Supporting Information Figure S7) but had more difficulty correctly assigning the other hybrid classes.

## 4 | DISCUSSION

This study showed that SAA broodstock and their offspring were readily distinguished from putatively wild trout in the seven catchments sampled. Moreover, wild fish were the dominant component of the sampled fish, even in catchments that had a long history of stocking. The exception to this was the PETT where fish of stocked origin accounted for 31.5% of the sample. This site is located high on a peat bog and natural spawning appears to be restricted to a small area of gravels at the outlet of Pettawater. Indeed, COLONY analysis indicated that only a very small number of redds may have contributed to the wild-spawned fry/parr population at this site.

The authors found that sea trout sampled in both marine and fresh waters were predominantly wild fish, with only a single sea trout (*i.e.*, 1.9% of sea trout analysed) being of purely stocked origin (Figure 2). Several sea trout, mainly caught in the vicinity of Weisdale Burn and the Burn of Sandwater, appeared to be hybrids between stocked and wild fish. All sea trout caught in Laxo Burn, which has been stocked annually since 2006, were apparently wild fish. Similarly, sea trout from the Burn of Arisdale (Yell), a stream that had not been part of the SAA stocking programme prior to 2015, were all wild fish. Despite extensive stocking over several years, there were generally very low numbers of stocked fish in the burns studied here. The exception was the PETT, where 31.5% of sampled fish (12/38) were of domesticated origin. The authors were also able to assign parentage for seven of these domesticated-origin fish (Table 2). Several factors that differ between stocked and wild-spawned fish, such as aggression, energy expenditure, predator avoidance and prior residency, have been suggested to affect the success of stocking programmes (Weber & Fausch, 2003); of particular importance is competition for resources such as territory and prey. The PETT has very little in the way of natural spawning gravels, so is likely to have only a small wild-spawned population. In the spring of 2015 (the year the authors sampled), SAA stocked the outflow stream from Pettawater with 5500 fry (Supporting Information Table S1a) and the

authors suggest that under lower levels of competition from resident, wild-spawned fish, relatively more of the stocked fry were able to survive, at least until the autumn of 2015. Thus, although juvenile fish of stocked origin were detected at nearly all sites studied (Figure 2), stocking appears to have had only a very limited impact on the wider Shetland sea trout run; additional analysis of larger sea trout samples is needed before final conclusions can be drawn.

There are two possible explanations for this study's findings: (a) stocked fish are either not smolting or are doing so in very low numbers. The migration phenotype (*i.e.*, resident *vs.* anadromous/migratory) has been shown to be under strong genetic control in several salmonid species (Ferguson *et al.*, 2019). Lemopoulos *et al.* (2019) found nine candidate single nucleotide polymorphisms associated with migration tendency in brown trout, whereas several genomic regions are associated with propensity to migrate in rainbow trout (*Oncorhynchus mykiss*; Hale *et al.*, 2013; Hecht *et al.*, 2013). The SAA hatchery stock used in Shetland are recorded as originating from the Howietoun strain of trout, and it is possible that they no longer possess the genetic variants necessary to facilitate the return to an anadromous life history. As reported in several recent studies (Archer *et al.*, 2019; Leitwein *et al.*, 2017), although non-anadromous trout populations do retain some of the genes associated with smoltification, in most cases, the smolts derived from non-anadromous populations showed a marked reduction in tolerance to sea water.

Alternatively (b), if stocked fish are smolting readily, they may be entering the sea when environmental conditions are sub-optimal and are experiencing higher levels of at-sea mortality than their wild counterparts. Timing of smolt migration is thought to be an adaptive trait, meaning smolts enter the marine environment at the "optimal" time. Marked differences in timing of ocean entry between wild and hatchery-origin chinook and Atlantic salmon have been found (Skaala *et al.*, 2019; Weitkamp *et al.* 2015) with hatchery-origin fish entering the ocean on average 22 days earlier than wild fish. Some Fraser River (British Columbia, Canada) populations of both chinook and sockeye salmon with early ocean entry have shown marked declines in productivity when compared to populations with smolts that enter the ocean up to 2 months later (Beamish *et al.*, 2013). These early smolting populations have lower growth rates, perhaps due to increased competition with early-year pink salmon smolts. Similarly, wild Victoria Island (Canada) chinook salmon smolts have 6–24 times higher survival than hatchery-origin fish in the Straits of Georgia (between Vancouver Island and British Columbia, Canada; Beamish *et al.*, 2012). Ruzzante *et al.* (2004) showed that in a region of Denmark with extensive stocking of domesticated brown trout, hatchery-origin sea trout experienced high at-sea mortality, resulting in very low numbers of domesticated-origin fish in the spawning component.

Results of both the STRUCTURE and NewHybrids analyses showed marked differences in levels of hybridisation between the putatively wild and domesticated trout in the burns that have been stocked (Figure 2). Weisdale Burn showed the highest levels of hybridisation, with several fish having genotypes suggestive of being advanced-generation hybrids. Conversely, other catchments showed much lower levels of hybridisation, *e.g.*, Laxo Burn, despite receiving

more than twice the number of hatchery-reared juveniles in the 10 years before this study's sampling of these streams (Supporting Information Table S1). Unfortunately, without data on numbers of wild fish present in these streams before stocking, it is not possible to assess the relative impact/proportions of the numbers stocked on a particular catchment. Nonetheless, the inter-catchment differences in levels of hybridisation suggest that the domesticated stock may have different levels of relative fitness in streams on the east and west of Mainland. Similar population-specific levels of introgression have been found in Danish brown trout populations that have been extensively stocked over long time scales (Hansen & Mensberg, 2009).

It is possible that the declines in the Shetland sea trout rod catch are linked to the collapse of sand eel (*Ammodytes* spp.) stocks around the islands in the late 1980s and early 1990s (as a result of overfishing for the manufacture of feed products for aquaculture; Furness, 2007). Similarly, overfishing for aquaculture feeds has been linked to the crash in the breeding success of seabird colonies on Shetland, specifically those species, such as puffins and Kittiwakes that, like sea trout, feed predominantly on sand eels (Furness, 2007). Sand eels support large populations of sea birds and other wildlife (Furness, 2007) and form an important part of the diet of sea trout, especially in coastal areas (Roche *et al.*, 2017). Closure of the Shetland sand eel fishery led to a partial recovery of both sand eel stocks and sea bird productivity (Furness, 2007), both of which also coincide with the increase in sea trout rod catch in the early 2000s.

Finally, the potential that the stocking programme may also have inadvertently acted to reduce the fitness of wild fish, as alleles associated with domestication introgressed into wild populations, also demands consideration. This phenomenon has been well studied in Atlantic salmon, where the deleterious effects of introgression from farmed fish are well recognised, often being associated with farm escapees (*e.g.*, Fleming *et al.*, 2000; McGinnity *et al.*, 2003). Similarly, although the effects of hybridisation and introgression from farm strains have also been studied in trout (*e.g.*, Pinter *et al.*, 2018), a particular focus of such studies has been the effects of alleles of domesticated origin on the fitness of fish in sea water, anadromy and marine survival. For example, Thrower *et al.* (2004) showed that rainbow trout that had been translocated above impassable waterfalls 70 years previously showed poor smoltification and low marine survival, whereas Phillis *et al.* (2016) carried out a common garden experiment involving below-barrier anadromous and above-falls river-resident populations of steelhead/rainbow trout (*O. mykiss*); analysis after 1 year showed that significantly more below-barrier smolts were detected moving downstream than above-barrier smolts, with above-barrier fish being 26% less likely to express the migratory tactic. Thus, although carried out with the aim of increasing fish numbers, it may be that nearly 20 years of stocking in Shetland inadvertently acted to reduce the numbers of trout undergoing smoltification, while also decreasing the marine survival of those trout which did go to sea. Ultimately, stocking may have acted only to delay the recovery of sea trout numbers in Shetland after the sand eel population recovered.

Overall, the results of this study provide an often-neglected qualification of the impact of stocking into wild populations and suggest that the recovery of the Shetland sea trout population was probably not directly linked to the SAA stocking programme; rather, improved marine survival of wild-origin sea trout as sand eels (a key food resource) recovered may have been the key factor. Irrespective, in 2017, in light of proposed restrictions on restocking detailed in a draft report to the Scottish Parliament on Wild Fisheries Reform (Thin *et al.*, 2014), which proposed that stocking be restricted to stock derived from broodstock native to the specific water being stocked (a.k.a. supportive-breeding), together with an impending ban on all stocking of fertile (diploid) trout (which came into force in in Scotland in 2020), the SAA took the decision to stop the programme. Accordingly, sea trout fishery improvement in Shetland will now focus on improving fish access to headwaters and clearing obstructions, such as redundant dams and weirs (Shetland Anglers Association, pers. comm.).

## AUTHOR CONTRIBUTIONS

R.A.K., A.L.M. and J.R.S. conceived the project and undertook fieldwork. R.A.K. conducted the molecular laboratory work and analysed the data. R.A.K. and J.R.S. wrote the paper with input from A.L.M.

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## SUPPORTING INFORMATION