Marine Scotland Science Report

The Scottish Government

Marine Scotland Science Report 23/12

# AN OVERVIEW OF POPULATION GENETIC STRUCTURING IN THE CROMARTY FIRTH FISHERIES TRUST

Prepared as part of the Focusing Atlantic Salmon Management on Populations (FASMOP) Project

Delivered in partnership with the Rivers and Fisheries Trusts of Scotland (RAFTS)

M W Coulson, S McKelvey, A Armstrong, E Cauwelier,

L Stradmeyer, J Gilbey, C Sinclair & E Verspoor

marinescotland science


Marine Scotland Science Report 23/12

# An Overview of Population Genetic Structuring in the Cromarty Firth Fisheries Trust

M W Coulson, S McKelvey, A Armstrong, E Cauwelier,
L Stradmeyer, J Gilbey, C Sinclair & E Verspoor

Prepared as part of the Focusing Atlantic Salmon Management on Populations (FASMOP) Project

Delivered in partnership with the Rivers and Fisheries Trusts of Scotland (RAFTS)

Published by Marine Scotland – Science

Marine Scotland is the directorate of the Scottish Government responsible for the integrated management of Scotland's seas. Marine Scotland Science (formerly Fisheries Research Services) provides expert scientific and technical advice on marine and fisheries issues. *Marine Scotland Science Report* is a series of reports that publish results of research and monitoring carried out by Marine Scotland Science. These reports are not subject to formal external peer-review.

Marine Scotland – Science
Freshwater Laboratory
Faskally
Pitlochry
PH16 5LB
Copies of this report are available from the Marine Scotland website at
www.scotland.gov.uk/marinescotland

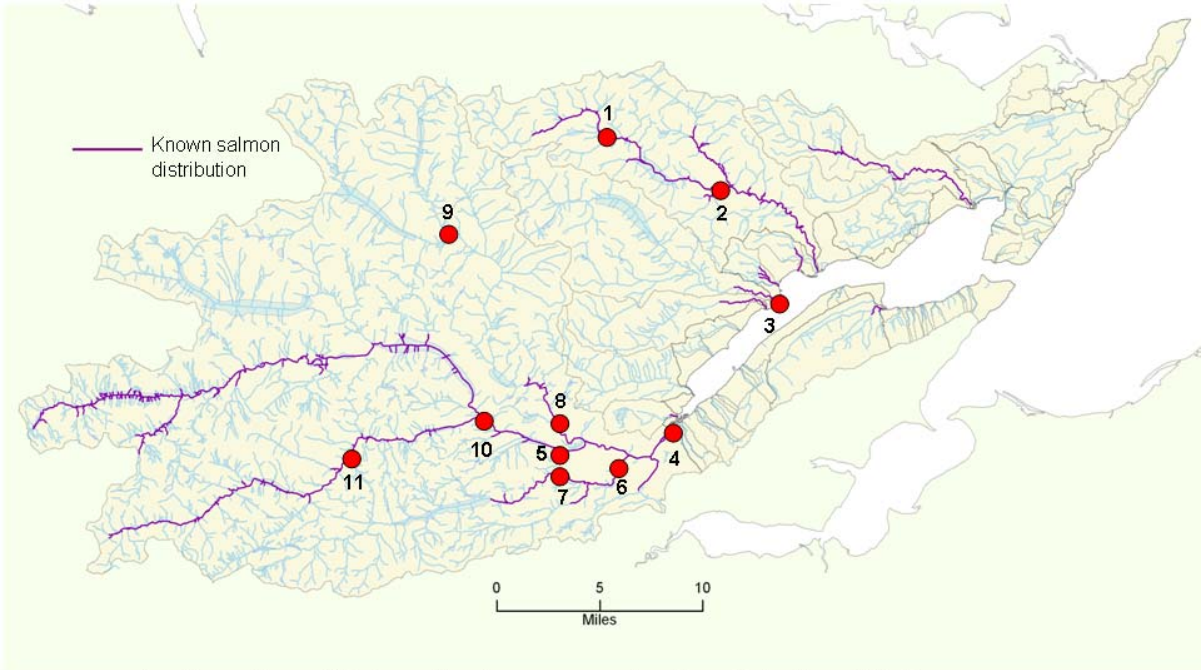
# An Overview of Population Genetic Structuring in the Cromarty Firth Fisheries Trust

M W Coulson[1], S McKelvey[3], A Armstrong[1,2], E Cauwelier[2], L Stradmeyer[2], J Gilbey[2], C Sinclair[1] & E Verspoor[2]

[1]RAFTS, Capital Business Centre, 24 Canning Street, Edinburgh EH3 8EG
[2]Marine Scotland Science, Freshwater Laboratory Faskally, Pitlochry, PH16 5LB
[3]Cromarty Firth Fisheries Trust, Aultgowrie Farm House, Aultgowrie, By Muir of Ord, IV6 7XA

## Executive Summary

This report describes how genetic information from juvenile Atlantic salmon sampled from 11 sites within the Cromarty Firth Fisheries Trust (Figure 1) have been analysed in order to help inform developing fisheries management activities. The key objective for the Trust was to define the genetic structure of the locations under investigation, in order to determine whether salmon in the area represent distinct breeding populations.




| | | | |
|---|---|---|---|
| 1 | Upper Alness (Abhain na Glasa) | 7 | Upper Orrin (Sawmill Cut) |
| 2 | Lower Alness (Boath) | 8 | Lower Blackwater (Contin) |
| 3 | Allt Graad | 9 | Upper Blackwater |
| 4 | Lower Conon (Dunglass Island Backwater) | 10 | Lower Meig (Scatwell Bridge) |
| 5 | Conon (above Blackwater junction) | 11 | Upper Meig (Strathconon) |
| 6 | Lower Orrin (Urray) | | |

**Figure 1**. A map of the Cromarty Firth, with sample sites that are involved in this report indicated in red with associated site names.

## Summary of findings

The analysis showed that most sites exhibited weak genetic differences from one another with the markers used, indicating low levels of genetic structuring among these sites. The Lower Orrin site was the only location different from all others. The remaining locations showed a mixture of results when compared to each other. For four locations, which were sampled for two year-classes there appeared to be mixed evidence of genetic signatures over time. While individual site-level analyses revealed weaker differences, greater differences were observed among groups of sites. These groups corresponded to: 1) the Alness & Allt Graad rivers, 2) a group of sites in the Conon that have all previously been stocked with Blackwater fish ('Blackwater Complex') and 3) a group of sites in the Conon that have not been stocked with Blackwater fish ('Wild Conon').

This weak degree of genetic differentiation observed among individual sites is largely reflected by the ability to predict where a sample is from using only genetic information (genetic assignment); where genetic signatures are strongly related to location, individuals are more likely to be assigned to the location from which they were originally sampled. The average value of correct assignment to site was only 43%, which is higher than one would expect if there was no genetic structure in the data. However, assignments conducted to the group level mentioned above were, on average, correct ~80% of the time. This level of analysis was used to assign 24 adult salmon (either rod-caught or captured at the Blackwater River trap) back to one of these three groups.

## Implications for management

The aim of the current FASMOP project was to identify distinct breeding populations of salmon within the Cromarty Firth. The results to date suggest that there are distinct breeding populations. However, the identification of these breeding populations to an individual site level is weaker than that observed to broader groups of sites. These broad groupings of sites allow for high accuracy of assignment of fish and may have implications for distinguishing fish from different rivers within the firth as well as stocked vs. wild components within the River Conon.

Finally, there are two possible reasons for the observed low levels of genetic structuring seen among individual sites:

- There is reproductive mixing of individuals between the different parts of the system. This could include possible stocking events in the past.
- The microsatellites in the study do not give the resolution required to adequately describe population structuring within the river.

The current genetic markers show overall weak genetic differentiation. However, this observation cannot be used to rule out the possibility of locally adapted traits being present within the system. This may be further clarified with the development and application of newer, more targeted, genetic markers. To determine if it is possible to improve assignments and gain better distinction for potential breeding populations, larger sample sizes and/or newer genetic markers will be required and possibly a more complete baseline of potential populations sampled.

## Introduction

Atlantic salmon (*Salmo salar* L.) are one of the world's most widely recognized and prized fish species. However, declines in numbers across much of the species' range have been cause for concern and the focus of intense management schemes and research efforts. Central to these efforts is the recognition of how the species is structured spatially across both broad and fine scales. Given the large native range encompassed by the species and their well-known ability to home to natal rivers, it is expected that Atlantic salmon will demonstrate a considerable degree of population structuring, representing discrete breeding units that are reproductively separated to varying degrees (Webb et al. 2007; King et al. 2007). This reproductive separation underlies the basis for locally adapted traits to establish across a widely variable environment. Indeed, ample evidence exists in favour of local adaptation in many salmonid species (Taylor 1991; Garcia de Leaniz et al. 2007; Fraser et al. 2011).

A principal tool used to resolve population structuring is the use of genetic markers. Such approaches have been used for decades, with most of the contributions towards salmonids occurring within the past 20 years (Verspoor, 2007). Initially, this work focused on range-wide patterns of differentiation (e.g. King et al. 2001; Verspoor et al. 2005) or documenting the expected structuring existing between different river systems (e.g. O'Reilly et al. 1996; Fontaine et al. 1997; McConnell et al. 1997; Spidle et al. 2003; Castric and Bernatchez 2004; Dillane et al. 2007). However, not as much is known about structuring within river systems and few examples exist for Scottish rivers (but see for example, Verspoor et al., 1991; Jordan et al., 2005).

Ecological studies have shown that different tributaries within a river may exhibit differences in traits such as run-timing (Stewart et al. 2002, 2006), variation in age at smolting (Englund et al. 1999) and sea-age at maturity (Niemela 2006), among others. When such differences are shown to have a genetic basis (e.g. Stewart et al. 2002, 2006), then salmon breeding in separate locations, for instance, above and below waterfalls or other natural features may often be heritably different in ways that affect their behaviour, survival and reproductive success. This may be true of neighbouring tributary populations and, since intermixing of these populations runs a risk of unknown magnitude, may not be desirable. Indeed, at its worst, mixing in vulnerable populations could have long term negative effects on population viability by reducing survival (McGinnity et al., 2003). Recent genetic analyses of Atlantic salmon have indicated that rivers may be structured on fine scales into multiple distinct breeding populations. Such studies have used both neutral genetic markers (Garant et al. 2000; Vaha et al. 2007; Dionne et al. 2008; Dillane et al. 2008) as well as markers for which there is an underlying basis for natural selection (Landry and Bernatchez 2001).

The suite of genetic markers used in the current survey are assumed to be “neutral” (meaning they are not known to be linked to heritable characteristics that may differ among locations such as run timing, growth rate, etc.). They will therefore largely reflect the shared ancestry of salmon among sites rather than make a direct assessment of the heritable trait characteristics that differ among them. Differences at such markers may *imply* that groups are sufficiently distinct for heritable trait differences to have evolved, however, where no difference is observed, we cannot rule out the possibility that these sites differ in heritable traits.

Given the recognition of the ‘population’ as a focal unit for management, it follows that knowledge of the genetic structuring among sites is required for certain management and conservation schemes. The potential for multiple, distinct populations to become established within a single river means that detailed knowledge needs to be gained regarding the scale at which such populations occur. As a first step in this process, a genetic baseline for systems needs to be constructed and built upon as more information becomes available.

In 2009, a partnership between the Rivers and Fisheries Trusts of Scotland (RAFTS), Marine Scotland Science (MSS), and the participating individual Fisheries Trusts and Boards was established. It set out to undertake a Scotland-wide survey of genetic structuring within all Scotland’s major salmon-producing rivers. This project, entitled **F**ocusing **A**tlantic **S**almon **M**anagement **O**n **P**opulations (FASMOP), had as its central aim to undertake a program of genetic sampling of Atlantic salmon stocks in river systems across Scotland. The purpose of this sampling scheme was to define the genetic structure among locations in order to determine whether salmon within and among the various systems in a given area represent distinct breeding populations. This work, alongside the EU SALSEA-MERGE and other MSS projects, is aiming to create a genetic map of salmon populations across Scotland, to help inform management and conservation efforts.

## Summary of Methods

Juvenile salmon from various locations within the area covered by the Cromarty Firth Fisheries Trust were sampled for genetic material by the Trust in order to inform fisheries management following methods outlined by Verspoor and Laughton (2008). Figure 1 shows the locations of the 11 sites that have been included in the genetic analysis for the Trust. Samples generally consisted of fry and/or parr (n= 47-50, depending on site) and for each individual, data from 17 genetic markers (microsatellites) were collected. The results from the microsatellite marker SsaF43 allowed us to identify any trout or

trout/salmon hybrids that may be present among samples. These individuals were then removed prior to analysis.

It is possible that samples are more reflective of families rather than populations, given the life-history stage(s) targeted by sampling and the potentially fine-scale geographic coverage (Hansen et al. 1997). This occurrence can alter the genetic signature of the sample and obscure population level differences. Therefore, prior to population level analyses, each site was screened for the presence of full-siblings, representing family groups and when identified, all but one individual of a full-sib family were removed. Additionally, this analysis can estimate how many breeders contributed to producing each sample, which may include contributions from precocious parr. Initial sample sizes as well as sample sizes after full-siblings were removed are presented in Table 1.

When samples sites included two life-history stages (i.e. fry and parr; Allt Graad, Lower Conon, Conon (BW junction), Upper Orrin), each of these sub-samples were initially tested for differences using the program CHIFISH (Ryman 2006). Where no significant differences were found, data from these sub-samples were combined; otherwise they were left separate for all further analyses. This resulted in 14 samples for subsequent analyses.

Data were then analysed using standard population genetic methods to evaluate the genetic relationships and groupings among the sample sites in order to obtain a general overview of population structure and address the objectives of the Trust.

A detailed methods and analysis section can be found in Appendix 1.

## Results

Broadly speaking, most sites exhibited weak or no significant genetic differences from one another, indicating low levels of genetic structuring among these sites with the current set of markers. The interpretation of the pattern and degree of differences in terms of the relationships among populations, combined with the known history and geographical proximity of sites can be useful to inform fisheries management decisions. Here we discuss the results of the FASMOP project summarizing the main genetic findings in terms of population genetic structuring within the Cromarty Firth.

### *Family effects*

A total of 545 juvenile salmon from the Cromarty Firth were involved in the genetic analysis. All sites were examined for family effects with relatively few samples being

removed due to full-sibling relationships (Table 1). The level of family effects differed between samples with the largest family group present in the individual samples ranging from 2 to 6 full-siblings and sample sizes subsequently being reduced by 2-28%. The most affected sites were the Upper Alness and the Lower Meig (Strathconon). Family effects were controlled for at each site before all further analyses. There was one trout and one salmon/trout hybrid sample identified among two locations (Table 1).

## Table 1

Details of samples used for this analysis, including original sample size, and details of each site following COLONY analysis to re-construct family relationships.

| Site | Site ID | Original sample size | Sample size analysed (sibs removed) | Number of breeders contributing to sample | Largest single family | Year sampled |
|---|---|---|---|---|---|---|
| Upper Alness | 1 | 50 | 36 | 47 | 6 | 2009 |
| Lower Alness | 2 | 50 | 45 | 60 | 3 | 2008 |
| Allt Graad† | 3 | 50 | 47 | 64 | 2 | 2010 |
| Lower Conon (Dunglass Island Backwater)† | 4 | 50 | 45 | 54 | 5 | 2008 |
| Conon (above BW junction)[1]† | 5 | 47 | 46 | 56 | 2 | 2010 |
| Lower Orrin (Urray) | 6 | 50 | 43 | 59 | 7 | 2008 |
| Upper Orrin (Sawmill Cut)‡ | 7 | 49 | 43 | 59 | 5 | 2008 |
| Lower Blackwater (Contin) | 8 | 50 | 45 | 60 | 3 | 2008 |
| Upper Blackwater | 9 | 50 | 46 | 65 | 2 | 2008 |
| Lower Meig (Scatwell Bridge)[2] | 10 | 49 | 36 | 52 | 4 | 2009 |
| Upper Meig (Strathconon) | 11 | 50 | 43 | 54 | 4 | 2008 |

1. One sample from this site was identified as a trout/salmon hybrid
2. One sample from this site was identified as a trout.

† Fry and parr were initially kept separate from this site as they were significantly different.

‡ Fry and parr from this site were combined as there was no significant difference between them.

*Population structuring*

Several sites were in the same year but for different age-classes (fry and parr for each of Allt Graad, Lower Conon, Conon above Blackwater junction, and the Upper Orrin). The CHIFISH analysis showed significant differences between the fry and parr from three of the four locations. Only the Upper Orrin site contained no differences between age-classes. Therefore the fry and parr from this site were combined, while they were kept separate initially for the other three sites. However, pairwise comparisons based on a measure of genetic differentiation ($F_{ST}$; Appendix 2) were not significant for any of the fry-parr comparisons. This discrepancy likely reflects differences in power between the two tests. Furthermore, some of these comparisons involved small sample sizes of one of the age-classes and/or unequal sample sizes between the fry and parr. Under such conditions, the tests implemented in CHIFISH may be unreliable (Ryman 2006). Pooling of small (<25) samples has been shown to produce more informative analyses of genetic structuring (Gomez-Uchida & Banks, 2005).

The genetic differences among sites show a small range in magnitude of genetic differentiation, with 82% (75 out of 91) of the pairwise comparisons being significantly different (Appendix 2). Among these comparisons, the Lower Orrin sample was the only sample that was different from all others based on pairwise measures of differentiation (Appendix 2). Most sites show a close genetic relationship displaying a mixture of significant and non-significant differences from one another. A visual representation of these relationships among locations can be found in Figure 2, which uses multi-dimensional scaling to represent pairwise estimates of genetic differentiation among sites (Appendix 2). Points which are closer together on the plot have a more similar genetic makeup while points further apart are more genetically discrete.

The MDS plot (Figure 2) shows a tendency toward several groupings. On the left side of the plot are the samples representing the two sites on the Alness as well as the Allt Graad. On the right-side of the plot are the remaining sites sampled from the Conon. Among these latter sites, there is a further split (on the second dimension) between two groupings: one contains the lower Conon and the Conon above the Blackwater junction, while the second is composed of the Upper Orrin, Lower Blackwater, Upper Blackwater, Lower Meig and Upper Meig. The Lower Orrin site is located somewhat between these two groups.

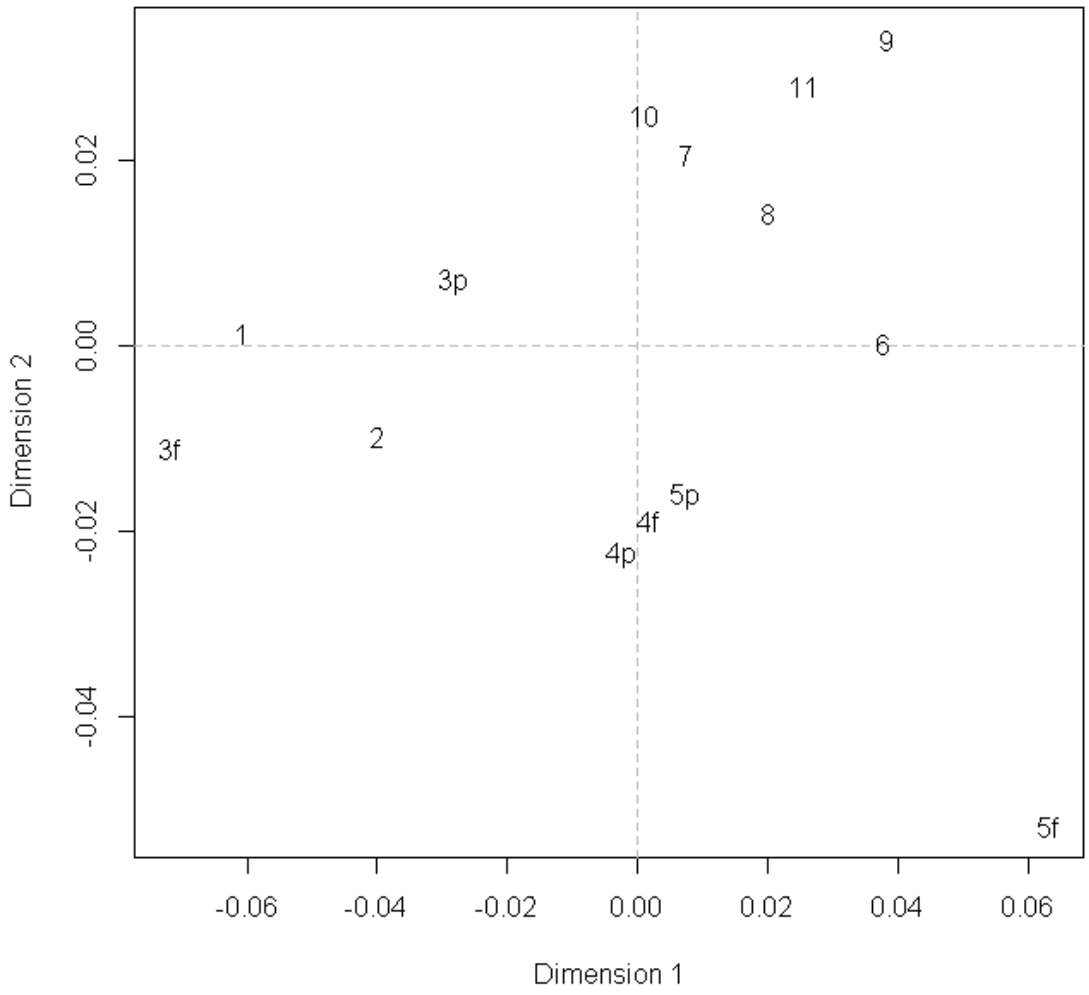
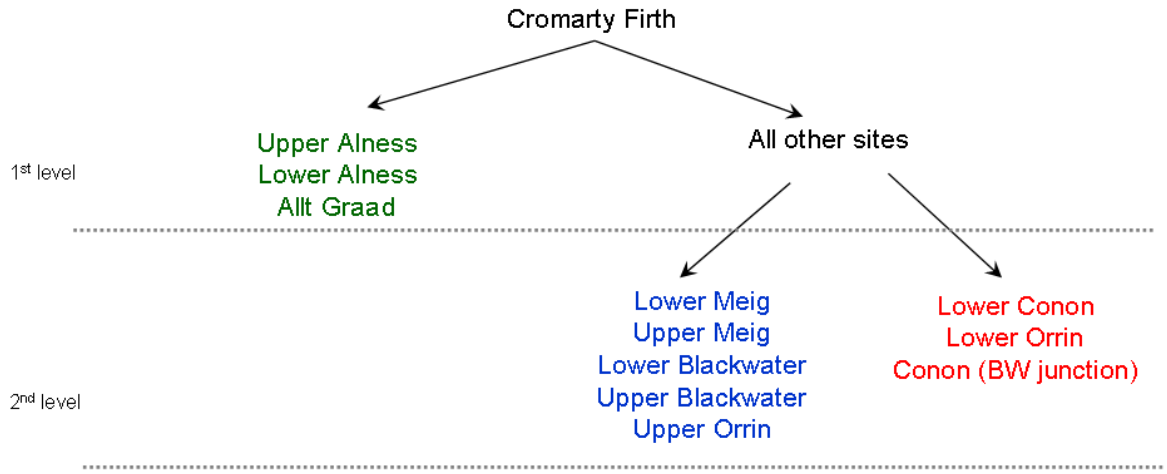



**Figure 2**. Multi-dimensional scaling (MDS) plot of genetic relationships among all sites based on pairwise estimates of genetic differentiation (Jost's D; see the appendix for details). Points which are closer together on the plot have a more similar genetic makeup while points further apart are more genetically discrete.

A clustering analysis that explores possible groupings of individuals other than the defined sampling sites was also carried out. This analysis was done in a hierarchical fashion, as larger genetic differences among groups may obscure weaker differences at smaller spatial scales. For each level, the analysis aims to determine from a given number of samples, the most likely number of groups and the membership of each individual into those groups. Each of the groups identified by the first round, are then separately analyzed in a second round and this process is repeated until further identification of groups is not possible. For the Cromarty Firth, at the broadest level, this analysis determined the most likely number of groups to be two. These two groups corresponded to: 1) a group composed of the Upper Alness, Lower Alness and Allt Graad, and 2) all sites on the Conon (Figure 3).

**(A)**




**(B)**

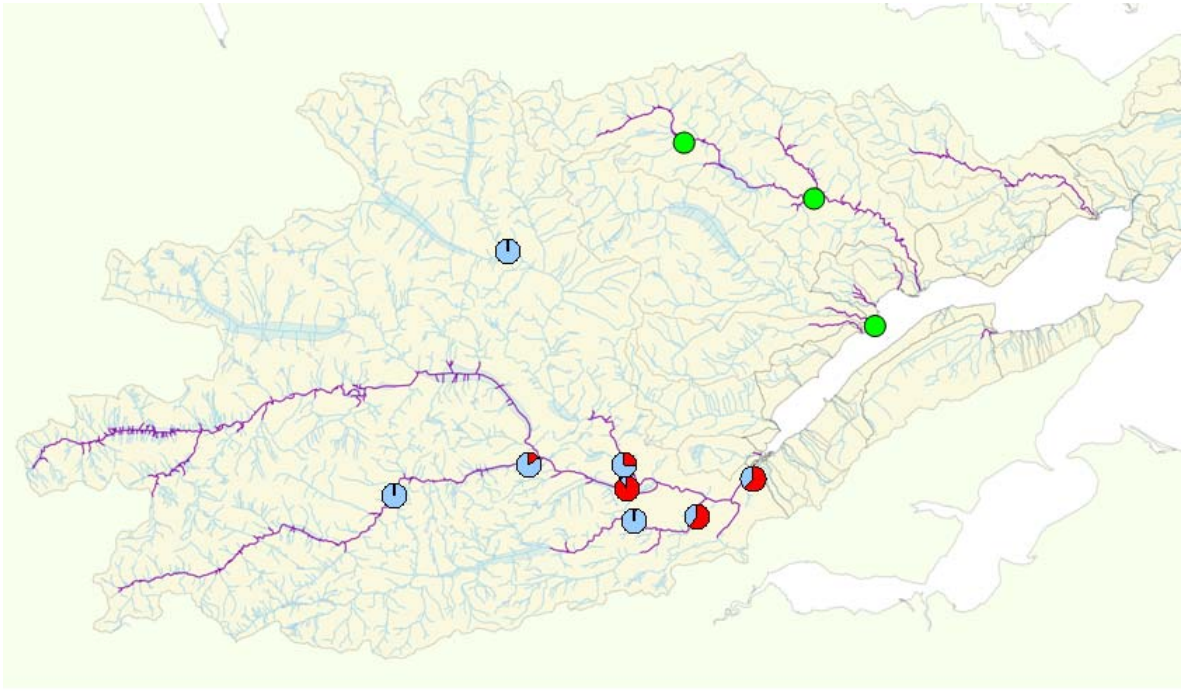


**Figure 3**. (A) Schematic summarizing the order in which the different groups were resolved according to the hierarchical clustering analysis. (B) Geographic representation of the relationships among sites, following the cluster analysis. Locations with the same colour are more similar to one another and belong in the same cluster. Among sites on the Conon, the two colours (red and blue) represent the proportional frequencies of the two groups at each of these locations.

The first group identified could not be split further by this method. However, among the second group, a second round of analysis revealed a further two groups. These corresponded to: 1) Lower Conon, Lower Orrin and Conon (above BW junction) and 2) Lower Meig, Upper Meig, Lower Blackwater, Upper Blackwater and Upper Orrin. It should be noted that the separation among these latter two groups was not as clear-cut as the first level. For several sites, most individuals contained a signal from both groups and membership was determined from the group displaying the highest proportion (see Figure 3B). A schematic summarizing the hierarchical groupings and a map showing the different group memberships is presented in Figure 3. Finally, it should be noted that when individual groups cannot be further separated, this does not necessarily mean, that there are not significant genetic differences. Rather, using this clustering approach, these smaller differences we observe are more difficult to tease apart and the distinction for splitting individuals into more than one group is less obvious.

*Genetic assignment of baseline*

The assignment analysis shows how useful this baseline genetic information is to identify which of the sampled sites a fish of unknown origin is from (Figure 4). Each individual fish is taken in turn and it is assessed from which of the sampling locations provided in the baseline, that individual is most likely to have originated. Assignment of fish back to their specific site of collection was, on average, correct 43% of the time (Figure 4A). While this average is greater than would be expected if assignments were purely random (11 sites, random = ~9%), this may reflect the weak population genetic structure underlying the data, but the magnitude of differences observed with the current markers among sites is not large enough to assign fish to location of sampling with higher accuracy.

An additional assignment level was investigated following the results of the clustering analysis. Given the definition of three groups, it was assessed how well individuals assigned back to each of these broader levels. For the two groups identified in the River Conon, individual sites were classified to whichever group composed more than 50% of the genetic make-up at that site. The five ‘blue’ sites (Fig. 3B) have all previously been stocked with Upper Blackwater fish and are therefore referred to as the ‘Blackwater Complex’. The three sites that were mostly ‘red’ are referred to as ‘wild Conon’ as these sites have never been stocked with Blackwater fish. Therefore the three groups (referred to as reporting groups) consist of: 1) Alness/Allt Graad rivers, 2) Wild conon and 3) Blackwater complex. Assignment of fish back to these three reporting groups was correct, on average, 78% of the time (Figure 4B). For both the Alness/Allt Graad group and the Blackwater complex group, assignment was ~80% but was lower for the wild Conon group (~60%).

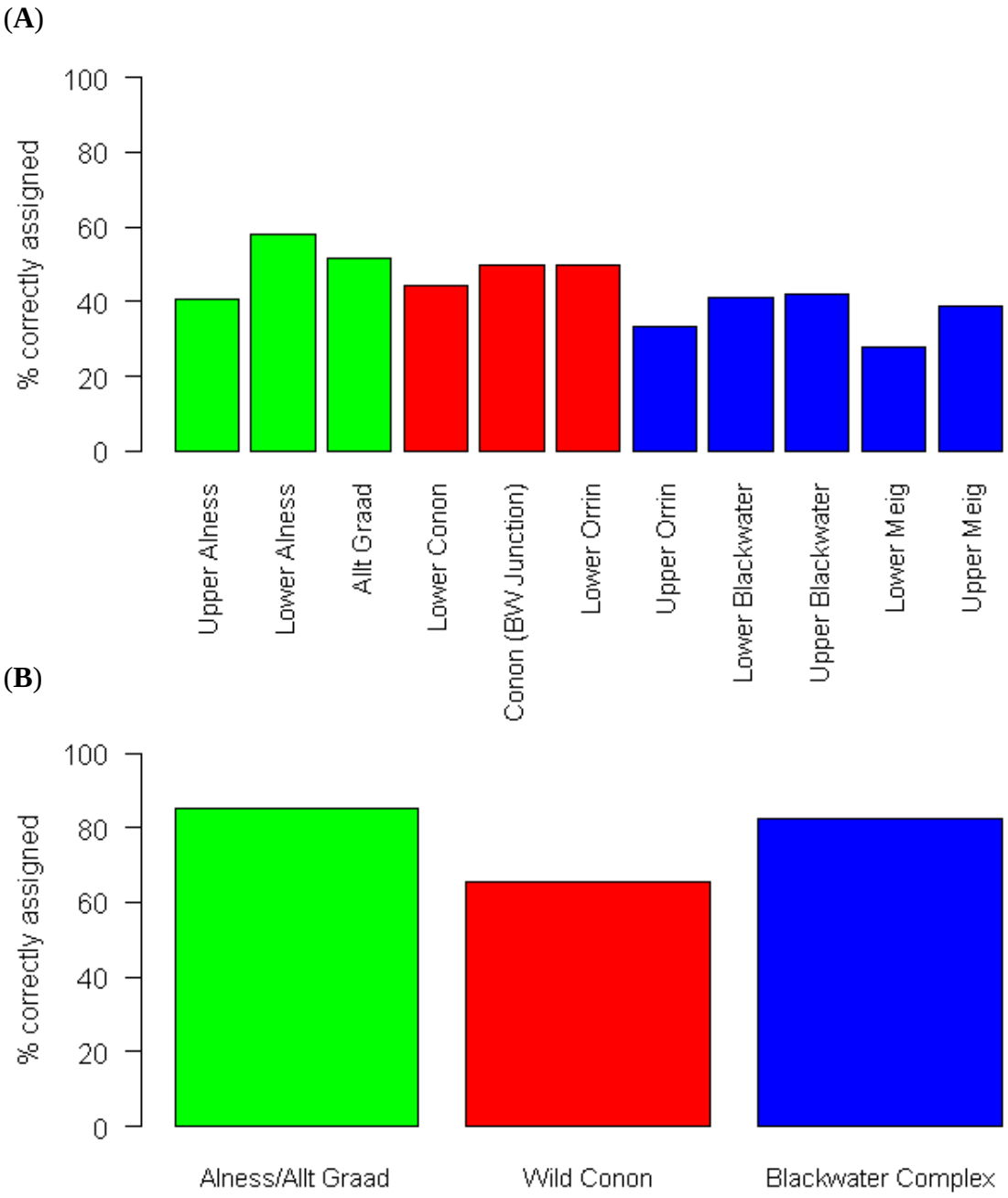



**Figure 4**. Percentage of fish sampled from each site that correctly assign back to (A) site level or (B) reporting group (see text for explanation).

It may be possible to improve assignment accuracy by implementing a cut-off rule for the probability that an individual gets assigned or by allowing individuals to not be assigned to any of the sites in the baseline (i.e. came from an unsampled population). Applying such a cut-off may come at a potential cost as not all fish in the baseline will be assigned (as some will have an assignment score lower than the minimal threshold). For the Cromarty Firth, a cut-off does not appear to improve assignments to site. For example, if we assign only fish that have a minimum of 70% assignment probability, overall correct

assignment to site is now 46% (compared to 43% previously) (Figure 5A). Even if a more stringent cut-off of 90% is taken, still only 53% of fish are correctly assigned. Additionally, due to this stringent cut-off, only 51% of the fish sampled are assigned (as about half the fish have an assignment score less than 90%). In other words, In other words, only ~50% of the fish in the baseline will be assigned and of those, only 50% are assigned to the correct site, indicating many fish are mis-assigning to site with high confidence. However, overall correct assignment to reporting group is 85% with a cut-off score of 90% assignment probability (Figure 5B). As seen in Figure 4B, even not applying a cut-off still resulted in 78% assignment to correct reporting group on average. However, with a cut-off applied, the assignment to the Wild Conon group increases the most and even at 70% assignment probability the correct assignment for this group is now 76% (versus 65.7% previously; data not shown). Therefore, depending on the specific cut-off value, between 80%-100% of the fish can be assigned to reporting group and at least 80% of those assigned will be correct. .

*Genetic assignment of adults*

Twenty-four adults were caught either by rod or at the Blackwater River trap. These adults were subsequently assigned using the juvenile samples as the baseline. For each adult, they were assigned to the most likely site and the scores for the top 5 sites were summed to determine the most likely reporting group. Unlike the juvenile baseline, the true origin of these fish is unknown but the plots in Figure 5 allow us to determine how accurate these assignments are likely to be. In other words, given the poor assignment to site-level and the observation of individuals mis-assigning with high assignment probability among the juveniles, we would expect ~50% of the site-level assignments to be wrong among the adults. However, as the reporting group level showed a much higher accuracy among the juveniles, the same expectation exists for the adults, in that we expect ~80% of the assignments to reporting group to be accurate. The assignment results to reporting group are summarized in Table 2. Among the eight adults captured at the Blackwater River trap, six were assigned to the ‘Blackwater Complex’ while the remaining two were assigned to the ‘Wild Conon’ group. Among the rod-caught adults, nine were assigned to the ‘Blackwater Complex’, six to the ‘Wild Conon’ and a single individuals was assigned to the ‘Alness/Allt Graad’ group. Most of the assignment scores were high (>90%), however several individuals still had low assignment scores (e.g. individual P1855 assigned to the ‘Wild Conon’ but only with 51.9% accuracy), suggesting some individuals were more difficult to assign to one group over another.

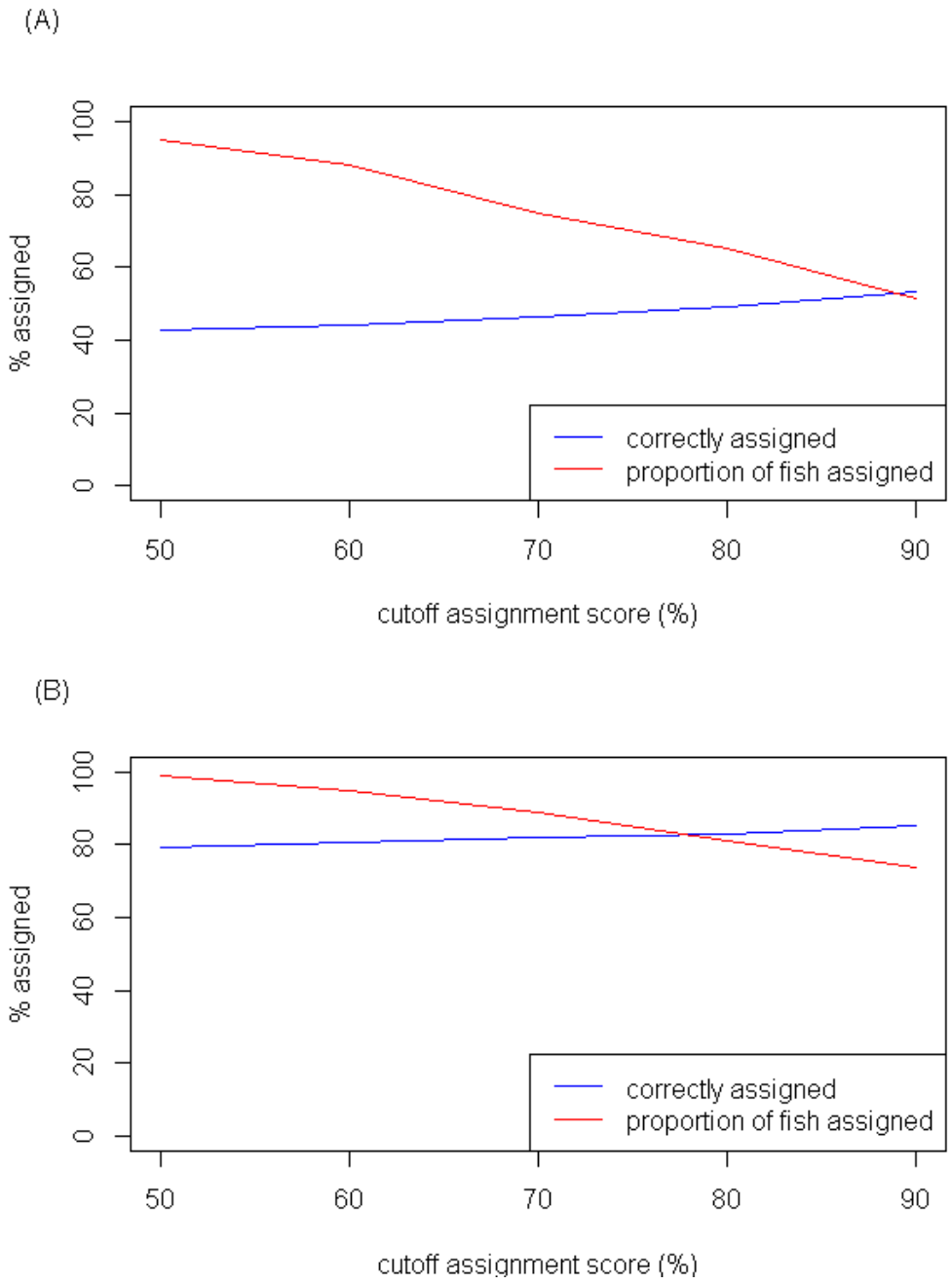



**Figure 5**. Plot of the percentage of fish assigned that were correct and the corresponding proportion of the total fish that were assigned for varying levels of assignment score cutoffs to (A) site level and (B) reporting group level (see text for details).

**Table 2** Assignment results of returning adults showing where they were caught, the date of collection, the reporting group to which they assign and the associated probability of assignment to that group.

| Individual | Sampled | Date of collection | Group assigned | % |
|---|---|---|---|---|
| P1868 | Blackwater trap | 10/12/2010 | Blackwater complex | 89.5 |
| P1869 | Blackwater trap | 10/12/2010 | Blackwater complex | 99.8 |
| P1870 | Blackwater trap | 10/12/2010 | Wild Conon | 93.2 |
| P1871 | Blackwater trap | 10/12/2010 | Blackwater complex | 100.0 |
| P1872 | Blackwater trap | 10/12/2010 | Blackwater complex | 100.0 |
| P1873 | Blackwater trap | 10/12/2010 | Blackwater complex | 99.3 |
| P1874 | Blackwater trap | 10/12/2010 | Wild Conon | 73.1 |
| P1875 | Blackwater trap | 10/12/2010 | Blackwater complex | 100.0 |
| P1859 | Lower Brahan | 05/08/2010 | Alness/Allt Graad | 99.1 |
| P1860 | Lower Brahan | 12/08/2010 | Wild Conon | 96.1 |
| P1861 | Lower Brahan | 17/08/2010 | Blackwater complex | 67.2 |
| P1862 | Middle Brahan | 04/08/2010 | Wild Conon | 100.0 |
| P1863 | Middle Brahan | 04/08/2010 | Blackwater complex | 99.8 |
| P1864 | Middle Brahan | 04/08/2010 | Blackwater complex | 95.8 |
| P1865 | Middle Brahan | 11/08/2010 | Blackwater complex | 74.4 |
| P1866 | Upper Brahan | 08/09/2010 | Wild Conon | 99.9 |
| P1867 | Upper Brahan | 08/10/2010 | Blackwater complex | 87.1 |
| P1851 | Upper Fairburn | 20/04/2010 | Blackwater complex | 99.8 |
| P1852 | Upper Fairburn | 24/07/2010 | Blackwater complex | 100.0 |
| P1853 | Upper Fairburn | 10/09/2010 | Blackwater complex | 91.3 |
| P1854 | Upper Fairburn | 16/08/2010 | Blackwater complex | 79.1 |
| P1855 | Upper Fairburn | 20/08/2010 | Wild Conon | 51.9 |
| P1856 | Upper Fairburn | 28/09/2010 | Wild Conon | 99.9 |
| P1857 | Upper Fairburn | 10/09/2010 | Wild Conon | 92.1 |

## Discussion

*Population structure*

The aim of the FASMOP project for the Trust was to identify distinct breeding populations of salmon within the Cromarty Firth. The results to date suggest that there are distinct breeding populations. While most locations were different from all others, larger groupings of sites displayed greater separation and were more easily defined, allowing for the application and evaluation of genetic assignment testing.

Four locations allowed for an assessment of the temporal stability of the genetic signature at those particular sites by comparing different age classes (fry and parr) collected in the same year. While three of the four showed evidence of temporal variation, this was only evident with the CHIFISH analysis and not based upon pairwise genetic differentiation values ($F_{ST}$). As noted earlier, this discrepancy likely is a result of the different power of the two tests and small sample sizes can influence the results, particularly of the CHIFISH tests. Therefore, while there appears to be mixed evidence for temporal variation at these four sites, these differences are generally smaller than those seen among sites. Whether this pattern holds throughout the system (or over longer periods of time) is still to be determined. This type of pattern supports the idea of weak meta-population structuring within the system, whereby spatially separated populations are connected by different degrees of interactions or exchange of individuals over time.

While most sites were significantly different from one another, the degree of these differences was generally weak. However, both the MDS plot and the cluster analysis revealed three broad groupings to which greater differences could be seen. Clearly, the largest separation was between the Conon and the Alness/Allt Graad rivers, which is perhaps not surprising given the homing abilities of Atlantic salmon to river of origin. However, within the Conon there was the further differentiation of a ‘Wild Conon’ vs. a ‘Blackwater Complex’ grouping. Thoses sites contained in the latter group have all previously been stocked from Blackwater fish at one point or another, while sites in the ‘Wild Conon’ group have never received stocked Blackwater fish. This finding suggests that stocking of Blackwater fish throughout parts of the system may have contributed to the homogenization of the genetic signatures at these locations. Furthermore, it is of interest to note that the Upper Blackwater site as well as the Upper Meig and Upper Orrin had no trace of ‘Wild Conon’ (Figure 3B) genetic make-up, while the remaining two sites had a small proportion. A similar pattern was observed among the ‘Wild Conon’ sites, whereby the mainstem Conon (above Blackwater junction) was mostly pure ‘Wild Conon’ group while the other two sites showed some affinity for both groups (but were mostly ‘Wild Conon’). This suggests the possibility of exploring the degree to which

individuals may be from one or the other of these groups, or indeed, whether they represent admixed individuals between wild and hatchery fish.

When there is clear evidence of distinct breeding populations, then a continued caution with respect to sourcing brood stock would be desirable in respect of stocking programmes. However, even with weak to little observed differentiation, the same caution should be exercised. As locations may still differ with respect to adaptive traits and until such issues can be addressed, then locally sourced brood stock should reduce the risk of disrupting any local adaptations that lead to increased survival. For instance, within these broad three groupings, the ability to separate different breeding populations is weaker. However, while a weak level of differentiation may be the result of more moderate exchange of spawning adults among sites, caution should still be used in making such an interpretation. If adaptive differences are present (e.g. for run timing behaviour), our neutral genetic markers will likely not have detected this. Therefore, other types of markers, which may be associated with adaptive traits, may help to further address the degree to which these locations represent distinct breeding populations.

*Genetic assignment*

The power to assign fish of unknown origin to their location of origin with high accuracy is possible where candidate locations show strong genetic differentiation. Such an approach is useful for discriminating the composition of mixed-stock fisheries. This could be useful, for instance, in assigning rod caught adults to their particular stock component. For example, it may be possible to use genetic assignments to determine whether salmon returning to a river at different time points are destined for different parts of the catchment if there is well defined structuring between these components and with genetic markers which may be associated with that particular trait. Genetic assignment allows one to calculate the probability that a given fish originated from a particular location. Then the location with the highest probability is taken as the site from where that individual originated. This is done for each individual and Figure 4 shows the proportion of individuals from a given site, which was assigned back to that site based on their genetic profile. If each location exhibits large differences from everywhere else, one would expect the accuracy of assigning individuals to the location from which they were sampled to be high (e.g. 90-100%). The average value of correct assignment to site is 43% (Figure 4A), which is higher than one would expect if there was no genetic structure in the data. While this supports the conclusion that there may be genetic differentiation among some locations, indicative of separate breeding populations, the data do not at present have the power to assign fish of unknown origin (e.g. rod caught adults) to their specific location with high accuracy. Even applying a strict cut-off (90% or better assignment score) for assigning fish still resulted in ~50% of fish being mis-assigned.

This indicates that approximately half the fish are being mis-assigned with high confidence scores.

Given the larger differences observed among the three reporting groups, it should follow that assignment to this level are much improved. Indeed, with no cut-off applied, correct assignment to reporting group was, on average, 78%. This could even be improved to 89% by applying a stringent cut-off score (90% or better) but has the caveat that only ~65% of fish will be able to be assigned. However, depending upon the particular question, this may be an acceptable loss to achieve the greater certainty among the fish that can be assigned. Not only do these results indicate relatively high accuracy in assigning to different rivers (or river groups) in the Cromarty Firth, but as well as in distinguishing between stocked and wild components within the Conon. Such an application could be of use in monitoring the relative success of hatchery versus wild fish. It is of note that a single adult captured in the lower Conon was assigned to the Alness/Allt Graad. Previous acoustic tagging results suggested a number of Alness fish spent time in the lower Conon (Stewart et al. 2009).

In order to improve assignments and gain better distinction for potential breeding populations, larger sample sizes and/or additional genetic markers may be required as well as a more complete baseline of potential populations sampled. As many assignments will try to assign individuals to sites represented in the baseline, if the 'true' site has not been sampled, fish from these missing sites will be forced to be incorrectly assigned. At present, these assignments represent our best estimates since all fish assigned were known to originate from sites in the baseline. For the most accurate and complete picture of assignments within a system, detailed knowledge of *all* breeding populations is required. It should be noted, however, that at certain geographical scales or for certain systems, assignment may not be possible with high accuracy, regardless of the samples and markers employed. If there is exchange of even modest amounts of spawning individuals over time between sites, then the genetic make-up of these sites will look relatively similar and prevent assignment to defined groups with high accuracy.

*Future work*

While there is evidence of genetic structuring within the Cromarty Firth, the level of resolution is most pronounced on a broader scale among groups of sites rather than among individual sites. Currently, the development and application of a different class of genetic marker (**S**ingle **N**ucleotide **P**olymorphisms, or SNPs) is underway in Scotland to address the resolution of population structuring in more detail and provide a more robust assessment. It is also likely that the use of SNPs could help to increase the assignment success to reporting group level even further. The use of SNPs offers at least two distinct advantages over the current suite of markers in that (1) the number of markers

screened for SNPs is much larger than that for microsatellites (100s - 1000s vs. 10s, respectively) and (2) that while microsatellites are selectively “neutral”, SNP markers should be associated with both “neutral” as well as actual traits, the latter of which some may be adaptive. The use of SNPs, either as an alternative to or in conjunction with microsatellites, has been shown to be promising for resolving different stock components with respect to fisheries management for various salmonid species (e.g. Narum et al. 2008, Glover et al. 2010, Beacham et al. 2010). Trying to target the underlying genetic differences that are associated with known biological (e.g. run-timing) or habitat (e.g. pH, elevation) differences will help to shed light on different stock components. For instance, finding a genetic marker associated with run-timing would allow for direct application toward the identification of spring vs. late-running stock components. This would allow for a more diagnostic application rather than using a set of random, ‘neutral’ genetic markers.

A number of factors may underlie population genetic structuring. At least one of these, touched upon here, is the potential impact played by stocking practices. Stocking in many areas has been common for Atlantic salmon both within and between systems. Such practices may influence why certain locations appear very distinct if they were sourced from a different location compared to the surrounding stock components. Alternatively, if stocking was widespread in an area, this could result in a more similar genetic make-up among stock components than would otherwise be the case. However, in order to address the degree, if any, to which stocking plays a role in genetic structuring, detailed knowledge of the stocking history and records are essential. Furthermore, including genetic samples from possible donor sources would provide an insight into whether those donors had made a lasting impact on the local stock. Additionally, the availability of historical samples that pre-date the stocking history of an area would be of particular value in addressing the impact as it would offer a comparison of the genetic make-up pre- and post-stocking levels of differentiation. Such an approach would be valuable for the Conon and the impact the Blackwater stocking has had on homogenizing other sites in the ‘Blackwater Complex’. Given the low levels of differentiation among ‘Blackwater Complex’ fish, this could be due, in part, to stocking. However, similarly low levels of differentiation were observed among the ‘Wild Conon’ fish and may be more an affect of the markers used and/or the biological and demographic relationships among locations within the system.

*Summary*

This analysis demonstrated overall weak levels of population structuring within the Cromarty Firth. The results suggest that there are distinct breeding populations within however, the degree of these differences is most apparent among groups of locations rather than among individual sites. However, at this group-level there appears to be

relatively strong resolution for the application of genetic assignment to aid in fisheries management questions. More work could help to clarify the extent and causes of the genetic structuring within the Cromarty Firth. This will likely involve the use of newer genetic tools and a more targeted approach to contribute to our overall understanding of the underlying salmon population structure and in turn, assisting the efficient management and conservation of this valuable resource.

## References


Beacham, T.D., McIntosh, B. and Wallace, C. 2010. A comparison of stock and individual identification for sockeye salmon (*Oncorhynchus nerka*) in British Columbia provided by microsatellites and single nucleotide polymorphisms. *Canadian Journal of Fisheries and Aquatic Sciences*, **67**, 1274-1290.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N. and Bonhomme F. 2004. GENETIX 4.05, logiciel sous Windows TM pour la genetique des populations. Laboratoire Genome, Populations, Interactions, CNRS UMR 5171, Universite de Montpellier II, Montpellier.

Castric, V. and Bernatchez, L. 2004. Individual assignment test reveals differential restriction to dispersal between two salmonids despite no increase of genetic differences with distance. *Molecular Ecology*, **13**, 1299-1312.

Crawford, N.G. 2010. SMOGD: software for the measurement of genetic diversity. *Molecular Ecology Resources*, **10**, 556-557.

Dillane, E., McGinnity, P., Coughlan, J.P., Galvin, P.T., Wilkins, N.P. and Cross, T.F. 2007. Spatial and temporal patterns in microsatellite DNA variation of wild Atlantic salmon, *Salmo salar*, in Irish Rivers. *Fisheries Management and Ecology*, **14**, 209-219.

Dillane, E., McGinnity, P., Coughlan, J.P., Cross, M.C., De Eyto, E., Kenchington, E., Prodohl, P. and Cross, T.F. 2008. Demographics and landscape features determine intrariver population structure in Atlantic salmon (*Salmo salar* L.): the case of the River Moy in Ireland. *Molecular Ecology*, **17**, 4786-4800.

Dionne, M., Caron, F., Dodson, J.J. and Bernatchez, L. 2008. Comparative survey of within-river genetic structure in Atlantic salmon; relevance for management and conservation. *Conservation Genetics*, **10**, 869-879.

Englund, V., Niemela, E., Lansman, M. and Heino, M. 1999. Variations in Atlantic salmon, *Salmo salar* L., smolt age in tributaries of the River Teno, Finland. *Fisheries Management and Ecology*, **6**, 83-86.

Evanno, G., Regnaut, S. and Goudet, J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology*, **14**, 2611-2620.

Fontaine, P.-M., Dodson, J.J., Bernatchez, L. and Slettan A. 1997. A genetic test of metapopulation structure in Atlantic salmon (*Salmo salar*) using microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 2434-2442.

Fraser, D.J., Weir, L.K., Bernatchez, L., Hansen, M.M. and Taylor, E.B. 2011. Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. *Heredity*, **106**, 404-420.

Garant, D., Dodson, J.J. and Benatchez, L. 2000. Ecological determinants and temporal stability of the within-river population structure in Atlantic salmon (*Salmo salar* L.). *Molecular Ecology*, **9**, 615-628.

Garcia de Leaniz, C., Fleming, I.A., Einum, S., Verspoor, E., Consuegra, S., Jordan, W.C., Aubin-Horth, N., Lajus, D.L., Villanueva, B., Ferguson, A., Youngson, A.F. and Quinn, T.P. 2007. Local adaptation. In: *Verspoor, E., Stradmeyer, L. and Nielsen, J.L. eds. The Atlantic Salmon: Genetics, Conservation and Management*. Oxford, Blackwell Publishing, 195-235.

Glover, K.A., Hansen, M.M., Lien, S., Als, T.D., Hoyheim, B. and Skaala, O. (2010) A comparison of SNP and STR loci for delineating population structure and performing individual genetic assignment. *BMC Genetics*, **11**, 2.

Gomez-Uchida, D. and Banks, M.A. 2005. Microsatellite analyses of spatial genetic structure in darkblotched rockfish (*Sebastes crameri*): is pooling samples safe? *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 1874-1886.

Hansen, M.M., Nielsen, E.E. and Mensberg, K.-L.D. 1997. The problem of sampling families rather than populations: relatedness among individuals in samples of juvenile brown trout *Salmo trutta* L. *Molecular Ecology*, **6**, 469-474.

Hubisz, M.J., Falush, D., Stephens, M. and Pritchard, J.K. 2009. Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, **9**, 1322-1332.

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.

Jordan, W.C., Cross, T.F., Crozier, W.W., Ferguson, A., Galvin, P., Hurrell, R.H., McGinnity, P., Martin, S.A.M., Moffett, I.J.J., Price, D.J., Youngson, A.F. and Verspoor, E. 2005. Allozyme variation in Atlantic salmon from the British Isles: associations with geography and the environment. *Journal of Fish Biology*, **67**, 146-168.

Jost, L. 2008. GST and its relatives do not measure differentiation. *Molecular Ecology*, **17**, 4015-4026.

Kalinowski, S.T. 2005. HP-Rare 1.0: a computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes*, **5**, 187-189.

Kalinowski, S.T., Manlove, K.R. and Taper, M.L. 2007. ONCOR a computer program for genetic stock identification. Montana State University, Bozeman. Available from www.montana.edu/kalinowski/Software/ONCOR.htm

King, T.L., Eackles, M.S. and Letcher, B.H. 2005. Microsatellite DNA markers for the study of Atlantic salmon (Salmo salar) kinship, population structure, and mixed-fishery analyses. *Molecular Ecology Notes*, **5**, 130-132.

King, T.L., Kalinowski, S.T., Schill, W.B., Spidle, A.P. and Lubinski, B.A. 2001. Population structure of Atlantic salmon (*Salmo salar* L.): a range-wide perspective from microsatellite DNA variation. *Molecular Ecology*, **10**, 807-821.

King, T.L., Verspoor, E., Spidle, A.P., Gross, R., Phillips, R.B., Koljonen, M-L., Sanchez, J.A. and Morrison, C.L. 2007. Biodiversity and population structure. In: *Verspoor, E., Stradmeyer, L. and Nielsen, J.L. eds. The Atlantic Salmon: Genetics, Conservation and Management*. Oxford, Blackwell Publishing, 117-166.

Landry, C. and Bernatchez, L. 2001. Comparative analysis of population structure across environments and geographical scales at major histocompatibility complex and microsatellite loci in Atlantic salmon (*Salmo salar*). *Molecular Ecology*, **10**, 2525-2539.

McConnell, S.K., O'Reilly, P., Hamilton, L., Wright, J.M. and Bentzen, P. 1995. Polymorphic microsatellite loci from Atlantic salmon (Salmo salar): genetic differentiation of North American and European populations. *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 1863-1872.

McConnell, S.K.J., Ruzzante, D.E., O'Reilly, P.T., Hamilton, L. and Wright, J.M. 1997. Microsatellite loci reveal highly significant genetic differentiation among Atlantic salmon (*Salmo salar* L.) stocks from the east coast of Canada. *Molecular Ecology*, **6**, 1075-1089.

McGinnity, P., Prodohl, P., Ferguson, A., Hynes, R., Maoileidigh, N.O., Baker, N., Cotter, D., O'Hea, B., Cooke, D., Rogan, G., Taggart, J. and Cross, T.F. 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.

Narum, S.R. 2006. Beyond Bonferroni: less conservative analyses for conservation genetics. *Conservation Genetics*, **7**, 783-787.

Narum, S.R., Banks, M., Beacham, T.D., Bellinger, M.R., Campbell, M.R., Dekoning, J., Elz, A., Guthrie, C.M., Kozfkay, C., Miller, K.M., Moran, P., Phillips, R., Seeb, L.W., Smith, C.T., Warheit, K., Young, S.F. and Garza, J.C. 2008. Differentiating salmon populations at broad and fine geographical scales with microsatellites and single nucleotide polymorphisms. *Molecular Ecology*, **17**, 3464-3477.

Niemela, E., Erkinaro, J., Julkunen, M., Hassinen, E., Lansman, M. and Brors, S. 2006. Temporal variation in abundance, return rate and life histories of previously spawned Atlantic salmon in a large subarctic river. *Journal of Fish Biology*, **68**, 1222-1240.

O'Reilly, P.T., Hamilton, L.C., McConnell, S.K. and Wright, J.M. 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 2292-2298.

Paterson, S., Piertney, S.B., Knox, D., Gilbey, J. and Verspoor, E. 2004. Characterization and PCR multiplexing of novel highly variable tetranucleotide Atlantic salmon (*Salmo salar* L.) microsatellites. *Molecular Ecology Notes*, **4**, 160-162.

Piry, S., Alapetite, A., Cornuet, J.-M., Paetkau, D., Baudouin, L. and Estoup A. 2004. GENECLASS2: A software for genetic assignment and first-generation migrant detection. *Journal of Heredity*, **95**, 536-539.

Pritchard, J.K., Stephens, M. and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945-959.

Rannala, B. and Mountain, J. 1997. Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 9197-9201.

Rousset, F. 2008. Genepop '007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103-106.

Ryman, N. 2006. CHIFISH: a computer program testing for genetic heterogeneity at multiple loci using chi-square and Fisher's exact test. *Molecular Ecology Notes*, **6**, 285-287.

Sánchez, J.A., Clabby, C., Ramos, D., Blanco, G., Flavin, F., Vázquez, E. and Powell, R. 1996. Protein and microsatellite single locus variability in Salmo salar L. (Atlantic salmon). *Heredity*, **77**, 423-432.

Spidle, A.P., Kalinowski, S.T., Lubinski, B.A., Perkins, D.L., Beland, K.F., Kocik, J.F. and King, T.L. 2003. Population structure of Atlantic salmon in Maine with reference to populations from Atlantic Canada. *Transactions of the American Fisheries Society*, **132**, 196-209.

Stewart, D.C., Smith, G.W. and Youngson, A.F. 2002. Tributary-specific variation in timing of return of adult Atlantic salmon (*Salmo salar*) to fresh water has a genetic component. *Canadian Journal of Fisheries and Aquatic Sciences*, **59**, 276-281.

Stewart, D.C., Middlemas, S.J. and Youngson, A.F. 2006. Population structuring in Atlantic salmon (*Salmo salar*): evidence of genetic influence on the timing of smolt migration in sub-catchment stocks. *Ecology of Freshwater Fish*, **15**, 552-558.

Stewart, D.C., Middlemas, S.J., Mackay, S. and Armstrong, J.D. 2009. Over-summering behaviour of Atlantic salmon, *Salmo salar* returning to rivers in the Croamrty Firth, north-east Scotland. *Journal of Fish Biology,* **74**, 1347-1352

Taylor, E.B. 1991. A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. *Aquaculture*, **98**, 185-207.

Vaha, J.-P., Erkinaro, J., Niemela, E. and Primmer, C.R. 2007. Life-history and habitat features influence the within-river genetic structure of Atlantic salmon. *Molecular Ecology*, **16**, 2638-2654.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P. and Shipley, P. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535-538.

Verspoor, E. 2007. Introduction. In: *Verspoor, E., Stradmeyer, L. and Nielsen, J.L. eds. The Atlantic Salmon: Genetics, Conservation and Management*. Oxford, Blackwell Publishing, 1-13.

Verspoor, E., Beardmore, J.A., Consuegra, S., Garcia de Leaniz, C., Hindar, K., Jordan, W.C., Koljonen, M.-L., Mahkrov, A.A., Paaver, T., Sanchez, J.A., Skaala, O., Titov, S. and Cross, T.F. 2005. Population structure in the Atlantic salmon: insights from 40 years of research into genetic protein variation. *Journal of Fish Biology*, **67**, 3-54.

Verspoor, E., Fraser, N.H.C. and Youngson, A.F. 1991. Protein polymorphism in Atlantic salmon within a Scottish river: evidence for selection and estimates of gene flow between tributaries. *Aquaculture*, **98**, 217-230.

Verspoor, E. and Laughton, R. 2008. Sampling river stocks of Atlantic salmon for genetic analysis to understand structuring into breeding populations. FRS Internal Report 2008.

Wang, J. and Santure, A.W. 2009. Parentage and sibship inference from multilocus genotype data under polygamy. *Genetics*, **181**, 1579-1594.

Webb, J., Verspoor, E., Aubin-Horth, N., Romakkaniemi, A. and Amiro, P. 2007. The Atlantic salmon. In: *Verspoor, E., Stradmeyer, L. and Nielsen, J.L. eds. The Atlantic Salmon: Genetics, Conservation and Management*. Oxford, Blackwell Publishing, 17-56.

Weir, B.S. and Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358-1370.

## Appendix 1

### Laboratory Procedures

DNA was extracted from individual fin clips using a standard proteinase K digestion (Fisher Scientific UK). The crude DNA extract was diluted (1 in 10) in 1xTE (Tris-EDTA) buffer for all further work. Seventeen microsatellite markers that have previously been developed for Atlantic salmon were amplified from each DNA extract by polymerase chain reaction (PCR) using fluorescently-labelled primers. The microsatellite markers used were: SP2201, Sp2210, SPG7, SP1605, SP1608, SP2216, SP3016 (Paterson et al., 2004), SsaD144, SsaD157, SsaD48, SsaD71 (King et al., 2005), Ssa14, Ssa289 (McConnell et al., 1995), Ssa202, Ssa171, Ssa197 (O'Reilly et al., 1996), SsaF43 (Sánchez et al., 1996). These 17 markers were amplified in three multiplex reactions according to the mixtures in Table 1 of this appendix. PCR reactions were conducted using the Type-it Microsatellite PCR kit (Qiagen). Cycle conditions were as follows : an initial denaturation at 95$^{o}$C for 5 min followed by 32 cycles of 94$^{o}$C for 30 s, annealing at either 58$^{o}$C (mix A and C) or 55$^{o}$C (mix B) for 90 s and extension at 72$^{o}$C for 60 s. After cycling, a final extension was completed at 60$^{o}$C for 30 min.

PCR products were run on a MegaBACE capillary sequencer (Amersham Biosciences) and compared against a size standard of Et Rox 550 (GE Healthcare) run along with each sample. Fragment sizes were scored with Fragment Profiler version 1.2 software (GE Healthcare). For data quality control, all results were independently checked by two people and in addition one in five results were scored “double-blind” and the results compared to calculate error rates.

### Data Analysis

The results from the microsatellite marker SsaF43 allowed us to identify any trout/salmon hybrids that may be present among samples, and also any mis-identified trout. The genetic information from these individuals was then removed from further analysis.

In order to remove bias in the data due to over-representation of family groups, an analysis of family relationships was performed using the software COLONY (Wang & Santure, 2009, Jones & Wang 2010) to identify full-sibling individuals. Furthermore, this analysis allowed for a prediction as to the number of breeders that contributed to each sample. For each location sampled, all but one member of a full-sibling group were removed from analysis.

**Table 1**

List of microsatellites used in the genetic survey with primer sequences, multiplex mixture, final primer concentration in the PCR and the reference reporting the microsatellite locus.

| Microsatellite marker | Sequence forward primers 5'-3' | Sequence reverse primers 5'-3' | Multiplex mixture | Final primer concentration (µM) | reference |
|---|---|---|---|---|---|
| Sp2201 | TTTAGATGGTGGGATACTGGGAGGC | CGGGAGCCCCATAACCCTACTAATAAC | A | 0.02 | Paterson et al., 2004 |
| Sp2210 | AAGTATTCATGCACACACATTCACTGC | CAAGACCCTTTTTCCAATGGGATTC | A | 0.02 | Paterson et al., 2004 |
| SPG7 | CTTGGTCCCGTTCTTACGACAACC | TGCACGCTGCTTGGTCCTTG | A | 0.02 | Paterson et al., 2004 |
| Ssa 202 | CTTGGAATATCTAGAATATGGC | TTCATGTGTTAATGTTGCGTG | A | 0.02 | O'Reilly et al., 1996 |
| SsaD144 | TTGTGAAGGGGCTGACTAAC | TCAATTGTTGGGTGCACATAG | A | 0.03 | King et al., 2005 |
| SsaD157 | ATCGAAATGGAACTTTTGAATG | GCTTAGGGCTGAGAGAGGATTAC | A | 0.03 | King et al., 2005 |
| Sp1605 | CGCAATGGAAGTCAGTGGACTGG | CTGATTTAGCTTTTTAGTGCCCAATGC | B | 0.015 | Paterson et al., 2004 |
| Sp1608 | AGCACACTCATCATCTTACCTAGAG | ATGGACAGAAAGATAATGAGGG | B | 0.015 | Paterson et al., 2004 |
| Sp2216 | GGCCCAGACAGATAAACAAACACGC | GCCAACAGCAGCATCTACACCCAG | B | 0.015 | Paterson et al., 2004 |
| Ssa171 | TTATTATCCAAAGGGGTCAAAA | GAGGTCGCTGGGGTTTACTAT | B | 0.015 | O'Reilly et al., 1996 |
| Ssa14 | CCTTTTGACAGATTTAGGATTTC | CAAACCAAACATACCTAAAGCC | B | 0.02 | McConnell et al., 1995 |
| Ssa289 | GTTTCTTTACAAATAGACAGACT | TCATACAGTCACTATCATC | B | 0.02 | McConnell et al., 1995 |
| Sp3016 | GACAGGGCTAAGTCAGGTCA | GATTCTTATATACTCTTATCCCCAT | C | 0.02 | Paterson et al., 2004 |
| Ssa197 | GGGTTGAGTAGGGAGGCTTG | TGGCAGGGATTTGACATAAC | C | 0.02 | O'Reilly et al., 1996 |
| SsaF43 | AGCGGCATAACGTGCTGTGT | GAGTCACTCAAAGTGAGGCC | C | 0.02 | Sánchez et al., 1996 |
| SsaD48 | GAGCCTGTTCAGAGAAATGAG | CAGAGGTGTTGAGTCAGAGAAG | C | 0.03 | King et al., 2005 |
| SsaD71 | AACGTGAAACATAAATCGATGG | TTAAGAATGGGTTGCCTATGAG | C | 0.03 | King et al., 2005 |

Where there was more than one site sampled within a 5-km distance, two life-history stages (i.e. fry and parr) sampled at the same site and/or a site was sampled in different years, the data were initially tested for differences using the program CHIFISH (Ryman 2006). Where no significant differences were found, data from these sites or time points were combined; otherwise they were left separate for all further analyses.

The program MICROCHECKER (Van Oosterhout et al. 2004) was used to screen for genotyping errors and non-amplifying variants (null alleles) in the raw data. In addition markers were checked for conformity to linkage equilibrium (probability test) and Hardy-Weinberg equilibrium (exact test), as implemented by GENEPOP version 4 (Rousset, 2008). In all cases, inference of significance was corrected for multiple-testing using the false discovery rate (FDR) method (Narum, 2006). Allelic richness is an estimate of the number of genetic variants found in a sample after controlling for sample size. This was calculated using the program HP-Rare (Kalinowski, 2005), and allows an assessment of differences in genetic diversity among samples standardized to a common sample size.

The genetic structure between groups was examined using two measures of genetic differentiation - pairwise $F_{ST}$ (calculated as $\theta$; Weir & Cockerham 1984) calculated in the program GENETIX (Belkhir et al. 2004) and assessed for significance with permutation tests using 500 randomizations. The second measure of differentiation, pairwise Jost's D (Jost, 2008) was calculated with the program SMOGD (Crawford 2010). A pairwise matrix of both distance measures is presented in Table 2 of this appendix. A multi-dimensional scaling (MDS) plot was drawn to illustrate the relationships among sites using the Jost's D measure of differentiation.

Clustering of individuals among potential distinct groups was undertaken with STRUCTURE 2.3.3, using the admixture model with correlated alleles (Pritchard et al., 2000). Briefly, this method assumes the number of distinct groups (*K*) in turn to be from 1 to some defined upper limit (i.e. the number of sites sampled). The analysis then determines which *K* is most consistent with the observed data,and assigns each individual to one of the defined groups. Furthermore, prior information on sampling sites was used to initiate the analysis with the LOCPRIOR option available in STRUCTURE 2.3.3 (Hubisz et al. 2009). A burn-in phase of 150,000 iterations was followed by a run phase of 250,000, using a minimum of five independent runs for each number of groups (*K*) being tested. Both the log-likelihood probabilities and the delta *K* method (Evanno et al., 2005) were examined to find the most likely *K*.

The utility of the data to assign fish of unknown origin to sample site was examined by running individual assignment tests using the program ONCOR (Kalinowski et al. 2007). Assignments were conducted using the method of Rannala & Mountain (1997). Location of assignment was taken as the site with the highest probability. Only individuals with a

complete multi-locus genotype (i.e. all 17 microsatellites) were chosen for assignment as comparing criterion values for individuals with differing number of markers typed is difficult (Piry et al 2004). Caution should be used when interpreting these results as the locations used may not represent the full spread of genetic diversity or populations present within the catchment, as well as differences in sample size, may affect the results.

## Appendix 2

Pairwise estimates of genetic differentiation among groups as defined in Table 1 (main text). Jost's D above diagonal, $F_{ST}$ below diagonal. Significant pairwise $F_{ST}$ values are indicated in italics and shaded in gray.

| | 1 | 2 | 3f | 3p | 4f | 4p | 5f | 5p | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | -- | 0.021 | 0.042 | 0.018 | 0.087 | 0.048 | 0.131 | 0.043 | 0.111 | 0.062 | 0.099 | 0.092 | 0.050 | 0.106 |
| 2 | *0.006* | -- | 0.003 | 0.006 | 0.063 | 0.065 | 0.103 | 0.014 | 0.084 | 0.068 | 0.074 | 0.087 | 0.062 | 0.061 |
| 3f | *0.010* | 0.002 | -- | 0.003 | 0.044 | 0.046 | 0.146 | 0.065 | 0.096 | 0.078 | 0.079 | 0.129 | 0.073 | 0.099 |
| 3p | 0.006 | 0.006 | 0.005 | -- | 0.049 | 0.021 | 0.099 | 0.011 | 0.075 | 0.030 | 0.050 | 0.044 | 0.050 | 0.042 |
| 4f | *0.021* | *0.019* | *0.016* | *0.012* | -- | 0.005 | 0.067 | 0.012 | 0.064 | 0.048 | 0.034 | 0.046 | 0.046 | 0.064 |
| 4p | *0.014* | *0.013* | *0.012* | 0.007 | 0.004 | -- | 0.050 | 0.001 | 0.037 | 0.038 | 0.016 | 0.062 | 0.016 | 0.062 |
| 5f | *0.033* | *0.026* | *0.033* | *0.022* | *0.017* | *0.013* | -- | 0.008 | 0.053 | 0.082 | 0.081 | 0.084 | 0.097 | 0.084 |
| 5p | *0.021* | *0.015* | *0.019* | *0.011* | 0.008 | 0.003 | 0.003 | -- | 0.026 | 0.028 | 0.005 | 0.015 | 0.016 | 0.025 |
| 6 | *0.023* | *0.015* | *0.019* | *0.016* | *0.015* | *0.011* | *0.014* | *0.011* | -- | 0.041 | 0.027 | 0.035 | 0.024 | 0.037 |
| 7 | *0.015* | *0.015* | *0.016* | *0.008* | *0.011* | *0.009* | *0.020* | *0.012* | *0.013* | -- | 0.007 | 0.003 | 0.004 | 0.003 |
| 8 | *0.019* | *0.015* | *0.018* | *0.011* | *0.007* | 0.004 | *0.016* | 0.005 | *0.006* | *0.005* | -- | 0.017 | 0.009 | 0.010 |
| 9 | *0.019* | *0.020* | *0.026* | *0.012* | *0.009* | *0.011* | *0.018* | 0.007 | *0.010* | 0.003 | *0.003* | -- | 0.017 | 0.006 |
| 10 | *0.014* | *0.014* | *0.015* | *0.011* | *0.010* | 0.004 | *0.022* | *0.010* | *0.009* | 0.004 | *0.005* | *0.007* | -- | 0.015 |
| 11 | *0.023* | *0.019* | *0.024* | *0.015* | *0.012* | *0.011* | *0.019* | *0.008* | *0.012* | *0.005* | 0.004 | 0.002 | *0.006* | -- |

Marine Scotland – Science
Freshwater Laboratory
Faskally
Pitlochry
PH16 5LB

Copies of this report are available from the Marine Scotland website at
www.scotland.gov.uk/marinescotland