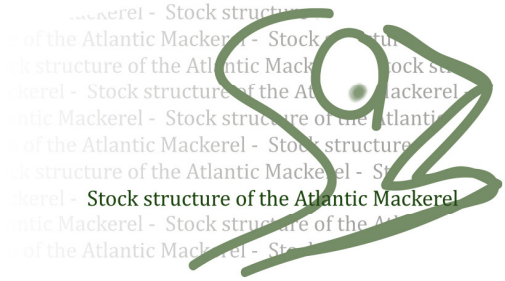

SAM: Stock structure of the Atlantic Mackerel (*Scomber scombrus* L.).

An ecological-time scale approach to solve stock(s) management

Marine resources strategic program

FINAL REPORT

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1. INTRODUCTION

The stock structure of North Atlantic mackerel *Scomber scombrus* L. is to date, not fully understood although biological differences are expected between populations located on both sides of the Ocean, but also at smaller geographical scale, e.g. within the eastern and western population(s). At the North-East side of the North Atlantic, the mackerel is currently divided into three distinct stocks for management purposes according to differences in spawning time: the North Sea stock which commonly spawns from May to July, the western stock which spawns west of Ireland and Scotland from March to July, and the southern stock which spawns north and west of Spain and in the Bay of Biscay from January to May (Figure 1) (ICES 2015).

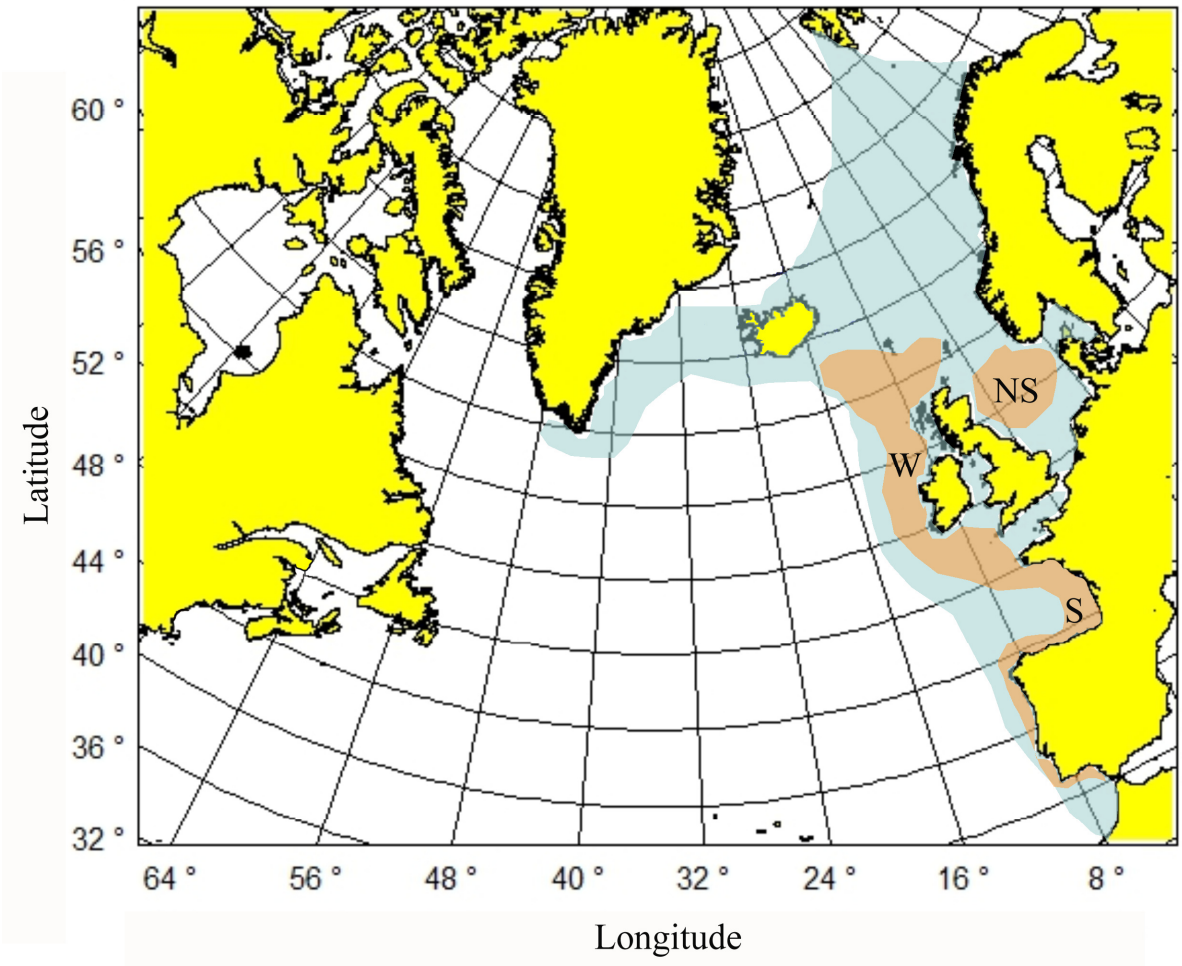

Figure 1. Spawning grounds of the European North Atlantic mackerel (*Scomber scombrus* L.) in the European waters (red) and feeding expansion distribution (blue) updated to 2014. W, Western spawning stock; S, Southern spawning stock; NS, North Sea spawning stock.

After the breeding season, the mackerel is known to exhibit long distance migration between spawning and feeding grounds (Figure 1). However, during recent years, the migration pattern of mackerel in European waters has changed dramatically both in timing and route. The feeding grounds

have gradually extended north into the Norwegian Sea and to the west into Icelandic waters, with mackerel located all around Iceland in the years 2008 and 2009 (Astthorsson *et al.*, 2012). From 2009 onwards, the European component of Atlantic mackerel has even extended further its feeding migration, up to Svalbard area in the North and to the East coast of Greenland in the West (Nøttestad *et al.*, 2015).

At the West side of the North Atlantic, the mackerel usually spawn off the American coast, from the latitude of Cape Hatteras, to the southern side of the Gulf of St. Lawrence. The spawning area used to cover almost the entire breadth of the continental shelf southward from Cape Cod, but it was confined more closely to the vicinity of the coast northward (Sette *et al.*, 1943). The occurrence of the mackerel in the Gulf of Maine was supposedly closely linked to the seasonal movements of the species as a whole. Concurrently to the feeding extension of the mackerel from the East side of the Atlantic to the North and West, the mackerel from the west side of the ocean has progressively disappeared from its spawning areas and was thought to migrate further North, both for spawning and feeding (Overholtz *et al.*, 2011; Radlinski *et al.*, 2013). One of the main hypotheses was that the Western mackerel had started to migrate further north for feeding following warm currents as the Eastern mackerel had done (Figure 2). Although, there are biological indications for a European origin, the Icelandic component has therefore been suggested to be a mixture of both North American and European stocks.

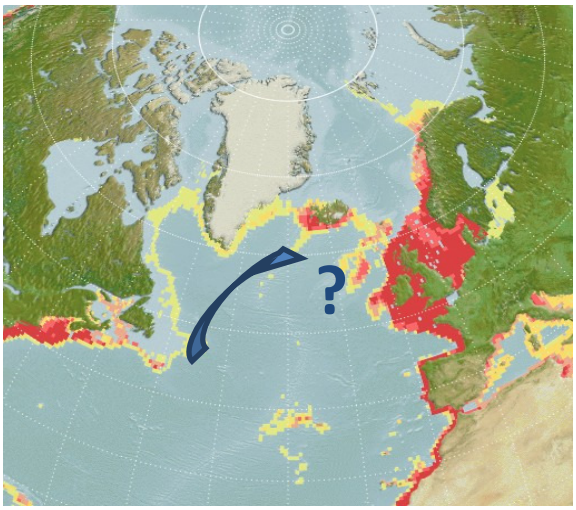

Figure 2. The relative probability of occurrence of Atlantic mackerel. (Source: http://www.aquamaps.org/ arrow inserted by authors).

The main objective of **SAM** was to build-up a large-scale network of skilled partners to develop genetic methods, which combined with other biological and environmental parameters, will address the origin of the mackerel stocks around Iceland, Greenland, Norway and Faroe Islands. **SAM** was therefore planning to develop new genetic tools as well as a new recommended approach for fisheries management, e.g. an ecological time scale approach (see Waples *et al.*, 2008), to better understand the dynamic of Atlantic mackerel in recent years and to implement it into fisheries management.

The project strategic objectives of SAM were:

- To develop new genetic markers using state of the art genome sequencing technique.
- Utilise the resulting genetic markers to analyse samples from different spawning and fishing grounds for mackerel around Iceland, Greenland, Norway, the Faroe Islands, Canada and adjacent waters for their genetic diversity and stock identification.
- To build a genetic database, including other biological and environmental data for Atlantic Mackerel stocks in the North Atlantic Ocean.

Expected outcomes of the project

The objective of **SAM** was to develop an efficient tool to correctly assess the origin of the Icelandic mackerel, by mapping and investigating the genetic characteristics of several stocks using newly developed genetic markers, such as microsatellite loci and Single Nucleotide Polymorphisms (SNPs), combined to other biological and environmental information (depth, temperature, distribution, abundance, morphological parameters). So far, despite the important issues raised concerning the stock quotas and origin of Atlantic mackerel in Icelandic waters, no genetic studies combined with existing data and life history portfolios have been performed on the targeted species. **SAM** wanted to lead to the establishment of a genetic map of mackerel in North Atlantic using several types of genetic markers combined to biological data, environmental data and survey data available. This objective was reached by developing a collaborative network with Nordic countries (within this application) for the establishment of a realistic sampling scheme and collaborative research plan.

2. MATERIALS AND METHODS

2.1 Sampling strategy (Work package 2)

The sampling strategy was developed to analyze a maximum number of samples available both at spawning and feeding grounds, and to statistically test whether genetic divergence could be detected among the spawning ground samples, and if a clear genetic assignment of the feeding grounds samples to the spawning ground samples could be performed (origin of the fish at feeding grounds).

A total of 6,622 samples were collected among which 1,917 collected at spawning grounds and 4,705 at feeding grounds (Table 1).

Table 1. Samples collected for the SAM project and genotyped at microsatellite loci and SNPs.

Atlantic Ocean	Unit/Stock	Locations	Year	Spawning	Feeding aggregation	Microsatellite DNA isolation	Microsatellite genotyping	SNPs DNA isolation	SNPs RADseq	SNPs genotyping
Western	Northern	Canada	2011	188		188	178	52	12	38
Western	Northern	Canada	2012	200		200				
Western	Northern	Canada	2013	200						
Western	Southern	Greenland	2011		8	200	200	38		6
Western	Southern	USA	2012	200		200	200	38		51
Eastern	Mixed	Greenland	2011		94	94	69			
Eastern	Mixed	Iceland	2010		299	250	127			
Eastern	Mixed	Iceland	2011		775	376	344	130	91	48
Eastern	Mixed	Iceland	2012		1,016					
Eastern	Mixed	Faroe Islands	2011		300	100	98			
Eastern	Mixed	Faroe Islands	2012		281					41
Eastern	Mixed	Norway	2011		59	59	59			
Eastern	Mixed	Shetland	2011		120	120	120			
Eastern	Western	Ireland	2012	199		199	187	30	19	40
Eastern	North Sea	North Sea	2009	31		31	31	31		
Eastern	North Sea	North Sea	2012		100					
Eastern	Southern	Bay of Biscay	2012	207		207	207	30	20	60
Eastern	Mixed	Greenland	2013		150					135
Eastern	Mixed	Greenland	2014							
Eastern	Mixed	Iceland	2013		325					
Eastern	Mixed	Iceland	2014		398					170
Eastern	Mixed	Faroe Islands	2013		200					
Eastern	Mixed	Faroe Islands	2014		147					
Eastern	Western	Ireland	2013	100						
Eastern	Southern	Spain	2013	100						47
Eastern	Southern	France	2013	69						64
Eastern	Western	Scotland	2013	100						
Eastern	Western	Ireland	2013	200						
Eastern	North Sea	North Sea	2013	75						46
Eastern	Mixed	Norway	2013		441					143
Eastern	Western	West Scotland	2013	48						48
North	Mixed	Jan Mayen	2013							95
				1,917	4,705	2,024	1,620	311	142	1,032
		Total collected			6,622					

Among these, samples were carefully selected for further processing with genetic markers. The selection was based on biological information and acute knowledge of migration and change in the distribution pattern of the species during the project.

2.2 Development of microsatellite loci (Work package 3)

Shotgun sequencing was carried out on a single individual of Atlantic mackerel caught in Icelandic waters in September 2011. DNA extraction, library construction, 454 FLX Titanium shotgun sequencing and the repeat detection by Flanker (Matis, Ltd.) have been published elsewhere (Libungan *et al.*, 2012). A total of 47,223,566 bases and 97,316 sequences were obtained with an average sequence length of 485 bases. Geneious Pro v5.5.6 (Drummond *et al.*, 2011) was used to design eighty primer pairs. Genomic DNA was isolated from gill or muscle tissue preserved in 96 % ethanol using AGOWA mag Midi DNA Isolation Kit (AGOWA Gmbh). Initial screening of 5 individuals using a tailed primer method (Schuelke, 2000) found 30 loci to be polymorphic and easily amplified. These loci were analysed on 46 samples from Icelandic fishing grounds (64,36°, -29,46°, April 2011) and 46 from Canadian spawning grounds (Gulf of St. Lawrence, June 2011) with dye labelled forward primers (Olafsdottir *et al*., 2013).

Polymerase chain reactions (PCR) were performed in a 10 µl volume containing 1.5–2.5 µl DNA (5–100 ng/µl), 0.80 µl of dNTP (10 mM), 0.75 U Taq polymerase (New England Biolabs Ltd.), 1 µl of 109 Standard Buffer (New England Biolabs Ltd.), 0.08–0.15 µl of a 50:50 ratio of labelled forward primer (100 µM) and reverse primer tagged on the 5'end with a GTTTCTT PIG-tail to enhance PCR quality (Brownstein *et al.*, 1996) and 1 µl betaine (5 M). PCR amplifications were as follows: 4 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 45 s at 58 °C and 45 s at 68 °C, and a final elongation of 7 min at 68 °C. Samples were analysed on an ABI PRISM 3730 sequencer using the GeneScan-500 LIZ internal size standard and genotyped with GeneMapper v4.0 (Applied Biosystems) (Olafsdottir *et al*., 2013).

ARLEQUIN v3.5 (Excoffier and Lisher, 2010) was used to calculate observed (H_O) and expected (H_E) heterozygosities and evaluate deviations from Hardy–Weinberg equilibrium (HWE). MICROCHECKER v2.2.3 (Van Oosterhout *et al.*, 2004) was used to investigate the cause of HWE departures. GENEPOP'007 (Rousset, 1998) was used to calculate linkage disequilibrium (LD) between pairs of loci, and sequential Bonferroni corrections for multiple tests were applied where appropriate (Rice, 1989).
Results obtained during this process were published in the International journal "*Conservation Genetic Resources*" (Olafsdottir *et al*., 2013).

Table 2 described the 30 microsatellite loci developed, their characteristics and their accession number in Genbank.

Table 2. Characteristics of the 30 microsatellite loci developed using 46 samples from Iceland and 46 samples from Canada. A_G, Allele range; N_A, number of alleles; H_O observed heterozygosity; H_E expected heterozygosity; AccNo, accession number of the primers in Genbank (http://www.ncbi.nlm.nih.gov/genbank/)(Olafsdottir *et al*., 2013).

Locus					Iceland		Canada		
name	**Primer Sequence (5'-3')**	**Repeat**	A_G	N_A	H_O	H_E	H_O	H_E	**AccNo**
Sscom04	F: ACACCAACAGCACAGTGACCC	AC	118-170	10	0.783	0.794	0.761	0.751	KC143306
	R: AGCCTTTGGCCATTGGAGACCC								
Sscom07	F: CCAACCCTCTTGTTCTTGCCTGGTG	AAAT	122-146	7	0.870	0.805	0.804	0.810	KC143307
	R: TGTCATCTTTGTTGAGCTGACTGGTG								
Sscom08	F: CGCTTCCTGTTTAGGCCCACGG	AC	136-160	11	0.630	0.637	0.370	0.441	KC143308
	R: GGCAAGTGTGTTGACTGCAGAGC								
Sscom10	F: AGCTGTCGTCATCACTCCTCATCC	ATC	160-187	10	0.826	0.731	0.739	0.777	KC143309
	R: GCAGCATGAGCCACAGCGAC								
Sscom11	F: ACGAGCCGAACGAGACACGC	AC	155-225	28	0.957	0.913	0.756	0.893	KC143310
	R: GGAGCTCTCGCTGTGTCTAACCG								
Sscom12	F: ATGAGCGGGCTTGGACGTGG	AC	164-194	12	0.767	0.809	0.933	0.811	KC143311
	R: AGTTTCCGTCAGCAGGACGCC								
Sscom13	F: CTGAGCCTGCCCACACGTCC	AC	171-179	4	0.522	0.537	0.543	0.506	KC143312
	R: TCTCCGTGAGACATGATAGGTTGGG								
Sscom14	F: TCAGGGTGGATCAAACCAGACCC	AC	184-200	8	0.761	0.750	0.689	0.791	KC143313
	R: CCGCATGTTGCTCTTTCGACCG								
Sscom16	F: TCCGCTAGCTGCATCACGCTC	AC	178-210	20	0.826	0.806	0.783	0.757	KC143314
	R: ACTCTCTGACAGTTTGTACATGCAGGG								
Sscom18	F: AAGGCCAGCCGTGCCACTTG	AC	192-272	39	0.864	0.953*	0.933	0.962	KC143315
	R: CATGCACACACGAGCACCAGC								
Sscom19	F: ACTGCAGCGGCGAACAAGCC	AC	204-230	9	0.717	0.758*	0.870	0.760	KC143316
	R: TGTGTCTGTTGCGCCCGACG								
Sscom23	F: GCGTGTGCAAGAAGTGGGCG	ATCC	208-244	10	0.826	0.806	0.870	0.835	KC143317
	R: TGCCAGTGACTGTGCGAGTGTG								
Sscom25	F: GCAGCAAACGCAACACTTGCC	AC	218-262	16	0.795	0.870	0.761	0.808	KC143318
	R: AGCTGGGCAAGCGGATCAAC								
Sscom28	F: AGCACAGGTGGCAGAGAGAGTG	AC	240-300	30	0.891	0.929	0.913	0.921	KC143319
	R: CGGCTTTAGCAGCACTATGTTTCAGTG								
Sscom30	F: GGACAAGCTGTCCACAATTAACTGGTC	AC	256-296	17	0.783	0.803	0.556	0.717*	KC143320
	R: GTGCTATCCATTGAGCTACTGTGCC								
Sscom32	F: TCCAGGATCAGGCTGAAAGCTGC	AC	234-316	24	0.891	0.863	0.870	0.861	KC143321
	R: GTGGCCGAGCGCTGAGCTG								
Sscom43	F: AAAGGCCAGCCGCACCAGTC	AC	100-118	9	0.783	0.723	0.826	0.724	KC143322
	R: AGTGCAGCTGCCGTCTTACTTG								
Sscom44	F: ACAGCACCGAGGATCCACCAG	AC	97-255	19	0.841	0.819	0.889	0.858	KC143323
	R: ACTGGAGCACACAGCTGACAGAC								
Sscom48	F: CGACAGTCTGGCACGGCAGG	AAG	129-162	10	0.761	0.786	0.822	0.849	KC143324
	R: TCCTCCATCTCCAACCACCTTGG								
Sscom49	F: GGTGGTACGGGTCATTTGTGTAGGTG	AACT	148-168	6	0.435	0.426	0.556	0.575	KC143325
	R: AGGACCGGAACTCCCTGCCTG								
Sscom50	F: CATCGCTTCTCATTCGCGCCC	AC	138-298	32	0.911	0.950	0.957	0.914	KC143326
	R: CCAGCCGGCTTGTTTCATGTTG								
Sscom51	F: CAGCTTGTTCAACCTTCAAATGTCTGC	AC	158-222	31	0.978	0.961	0.978	0.962	KC143327
	R: TGACAAGCGTCGACTCGTCTTCC								
Sscom52	F: TGGTCCCAGCCAATTGCAGAGC	AC	167-183	9	0.848	0.707	0.761	0.699	KC143328
	R: AAGGCCCTTGCTGGTTGCCC								
Sscom55	F: TGTCTCGCTCCTGCCTCCATC	AC	180-248	26	0.907	0.910	0.870	0.945	KC143329
	R: CGACGGGCTGCCTCTCTGTG								
Sscom56	F: CGCTGCTGACAGAGACGGTGC	AC	210-296	30	0.930	0.945	0.897	0.920	KC143330
	R: TCCCGGGAACGTTAGCGCAAG								
Sscom57	F: ACAGTCCAACCACAACGAGAGGG	AC	202-222	8	0.667	0.707	0.739	0.715	KC143331
	R: TGGACAGGCTATTGGGCTCGC								
Sscom58	F: AGATCGGCTGCTGGCATCGC	AAC	197-275	23	0.829	0.933*	0.717	0.918*	KC143332
	R: GCTCCTGCAGTGGGAACGGG								
Sscom62	F: AAGCCCTCAAATGCCTGTTTCTGT	ACTC	231-255	6	0.609	0.593	0.543	0.561	KC143333
	R: AACCTGCCTGCAATCAGCACA								
Sscom66	F: TGCACAGTAAGGTGGATAGGGACTTG	AC	269-295	11	0.658	0.687	0.696	0.741	KC143334
	R: TGGAAGTGAACGCTAGCTGATGC								
Sscom69	F: ACCCGGACTGCCGTACACAC	AC	279-349	28	0.822	0.874	0.690	0.860*	KC143335
	R: CCCAAACACTTAAGACTCCAGCTCAC								

2.3 Development of Single Nucleotide Polymorphisms (SNPs) (Work package 3)

A total of 20 individuals from each of the sampled baseline populations (France, Ireland, and Canada) were used to construct a single read RAD sequencing library. In addition, 90 individuals from a feeding aggregation sample in Icelandic waters were also sequenced. A single individual was first sequenced in high depth using paired end sequencing. RAD sequencing was carried out using a modified protocol based on Baird *et al.* (2008). Briefly DNA was extracted using QIAGEN Blood and Tissue kits, quantified using Qubit DNA assay, and standardised to 30ng/µL. Five hundred nanograms of each sample was then digested with 5 units of SbfI followed by ligation of barcoded Illumina adapters complementary to the restriction overhang. Samples were pooled and sonically sheared using a Bioruptor UCD-200 Standard Sonication Device (Diagenode). DNA ranging from 350 to 550 bp in size was separated using agarose gel electrophoresis, excised and extracted with the QIAquick Gel Extraction Kit. The size-selected DNA was ligated to P2 adapters and PCR amplified to select for fragments containing the 5′ and 3′ sequences necessary for Illumina sequencing, which was carried out on a Hi-Seq 2500 at Floragenex (USA) (Figure 3).

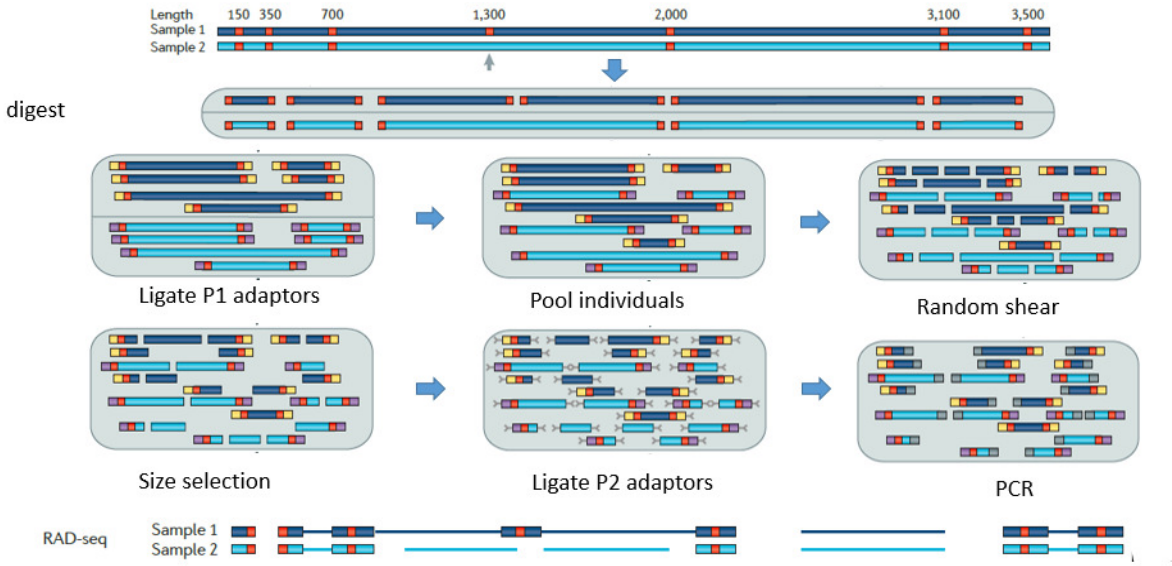

Figure 3. Schematic of RAD sequencing, showing all the steps from the initial enzyme digestion to the final sequencing of the PCR products for 2 individuals. Enzyme cut site, red; adaptors, yellow and purple (Davey *et al.*, 2011).

A threshold of 2,000,000 reads per sample was set to ensure high quality per sample. Polymorphic SNPs were identified from the single read data using STACKS (Catchen *et al.*, 2011) and SAMTools (Li, 2011). Data filtering consisted of 2 main steps, the first removed contigs with low depth, low sequence quality, and high numbers of flanking SNPs. The second removed loci with high levels of missing data, poor quality genotypes, and a minor allele frequency (MAF) below 5%.

Initial analyses were carried out on the full single read dataset to identify population structure present with a dataset not under strong selective pressure, assays were developed for SNPs that showed high levels of assignment power. The single read sequences were then assembled together with the paired end reads to provide sufficient flanking sequence surrounding each SNP to allow the design of assays to allow the SNPs to be genotyped on the Fluidigm Biomark HD (Figure 4). Assays were designed using the Fluidigm D3 assay design online software (https://d3.fluidigm.com).

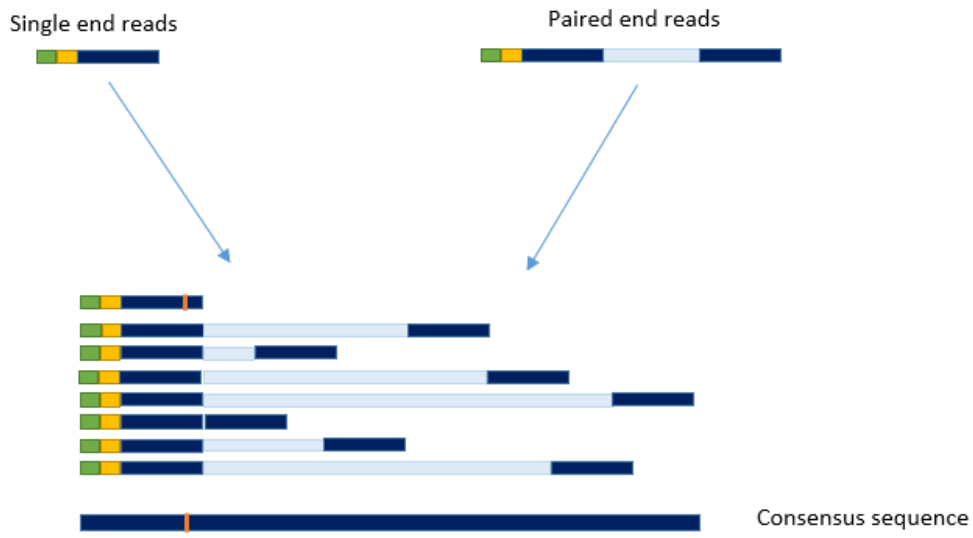

Figure 4. Assembly of single- and paired-end reads, demonstrating how the sequences were aligned to produce long enough flanking sequences for the development of the Biomark assays (added barcode, green; enzyme cut site, orange; location of SNP in single read data, yellow; sequenced DNA region, dark blue; unsequenced DNA region, pale blue).

2.4 Microsatellite loci genotyping (Work package 4)

DNA was isolated from all samples using the AGOWA mag Midi DNA Isolation Kit (AGOWA Gmbh) following the manufacturers protocol. DNA quality and quantity was determined with a NanoDrop Spectrophotometer (Thermo Fisher Scientific Inc.) prior to genotyping. A total of fourteen microsatellite loci were genotyped (*Sscom04, Sscom07, Sscom08, Sscom10, Sscom25, Sscom43, Sscom48, Sscom49, Sscom50, Sscom52, Sscom55, Sscom57, Sscom62 and Sscom66*; Olafsdottir *et al.,* 2013).

PCR reactions were performed in a 10 µl volume containing 10–50 ng DNA, 200 µM of each dNTP, 0.75 U Taq polymerase (New England Biolabs Ltd.), 1 µl of 10x Standard Buffer (New England Biolabs Ltd.), 0.3-2.5 µl of a 50:50 ratio of labelled forward (100 µM) and reverse (100 µM) primer tagged on the 5'-end with a GTTTCTT PIG-tail (Brownstein *et al*., 1996). 1 µl of betaine (500 mM) was added to enhance DNA amplification if needed. PCR reactions were performed on a Tetrad2 Peltier thermal cycler (BioRad), and cycle conditions corresponded to those described in Olafsdottir *et al*., (2013). Samples were analysed on an ABI PRISM 3730 sequencer using the GeneScan-500 LIZ size standard and genotyped with GeneMapper v4.1 (Applied Biosystems).

2.5 SNPs genotyping (Work package 4)

In addition to the individuals sequenced for each population, a large number of additional individuals were then genotyped using the Fluidigm Biomark™ system for the 96 SNPs with successful assay designs. These samples are details in Table 3.

Table 3. Location and years of samples collected for the SNPs sequencing and genotyping (Also presented in Table 1). Numbers between brackets represent the number of samples available.

location	year	Number successfully sequenced (total)	Number successfully genotyped (total)
Greenland (W)	2011	-	6 (8)
Canada	2011	12 (20)	37 (40)
Faroes	2012	-	40 (48)
France	2012	20 (20)	30 (30)
France	2013	-	43 (48)
Ireland	2012	19 (20)	20 (30)
Ireland	2013		46 (48)
Iceland (SE)	2011		47 (48)
Iceland (NE)	2014		80 (96)
Iceland (SE)	2014		48 (48)
Iceland (W)	2011	40	-
Iceland (NW)	2011	51	-
Greenland (SE)	2014	-	40 (48)
Greenland (SE)	2014	-	47 (48)
Greenland (S)	2014	-	48 (48)
North Sea	2013	-	46 (48)
North of Jan Mayen	2013	-	95 (96)
South Norway	2013	-	48 (48)
Norway	2013	-	25 (27)
Norway (coastal)	2013	-	40 (45)
North Norway	2013	-	30 (30)
West Scotland	2013	-	48 (48)
North Spain	2013	-	47 (48)
USA	2012	-	51 (56)

Standard Biomark protocols were followed (User guide: PN 68000098 N1). Briefly, Pre-amplification PCR reactions were first carried out for the samples, in this step all the forward (Specific Target Amplification, STA) and reverse primers (locus specific primer, LSP) for the panel of 96 SNPs were multiplexed in a single PCR. This step removes the need to standardize DNA concentrations prior to PCR amplification, and ensures good genotyping success with poor quality samples. This PCR was carried out in 5 µl volumes (1.25 µl of genomic DNA, 2.5µl of 2X Multiplex PCR Master Mix (QIAGEN), 0.5 µl of 10xPrimer pool (0.5 µM each SNP primers) and 0.75 µl PCR water; PCR cycles

were 95°C for 15 min followed by 14 cycles of 95°C for 15 sec, 60°C for 4 min), and post-PCR product (PreAmp DNA) was diluted 1:100 with dH_2O prior to genotyping.

Multiplex SNPs genotyping was conducted using SNPtype Genotyping Assays in Fluidigm® 96.96 Dynamic Arrays using standard methods (User guide: PN 68000098 N1). Each array was loaded with 94 samples, one negative control (H_2O) and one positive control (a DNA mixed to aid with the identification of heterozygotes). The PCR was carried out in nL volumes, with 5 µl of each 96 SNPtype Assays were loaded on the right of the array (7.5 µM allele specific primers (ASP1 and ASP2, forward primers with sequence tags attached, one for each fluorophore) and 20 µM LSP, 2X Assay Loading Reagent and PCR grade water); Sample Assays were loaded on the left of the array (5 µL total volume; 2.5 µl Biotium 2X Fast Probe Master Mix (Fluidigm), 0.25 µl 20X SNP Type™ sample loading reagent (Fluidigm), 0.083 µl 60X SNP Type reagent (Fluidigm), 0.03µl ROX™ (Life Technologies), PCR grade water and 2.1 µl of the diluted PreAmp DNA). The arrays were primed and loaded with the 96.96 IFC controller, and after loading, the chip was placed in the a BioMark™ HD System (Fluidigm) for PCR cycling according to manufacturer‘s instructions. After amplification the Dynamic Arrays were read on the BioMark™ HD and scored using Fluidigm® SNP Genotyping Analysis software.

2.6 Statistical analyses of the microsatellite loci (Work package 4)

All markers were checked for null alleles and potential genotype scoring errors with Microchecker (Van Oosterhout *et al.*, 2004). Genetic diversity indices including the number of alleles (n_a), observed (H_O) and expected (H_E) heterozygosities, and departure from HWE were calculated in ARLEQUIN (Excoffier and Lisher, 2010). Linkage disequilibrium was tested for between loci, and within each population in ARLEQUIN (Excoffier and Lisher, 2010) with a Markov Chain (MC) of length 10^6 and 100 000 dememorizations. A false discovery rate (FDR) was calculated to correct for multiple testing using the approach by Benjamini and Yekutieli (2001). The three samples from France were tested for pairwise genetic differentiation using the unbiased F_{ST} estimator (Weir and Cockerham, 1984), to enable pooling of these samples for further analyses (Table 4). Statistical significance was assessed using permutation tests implemented in GENEPOP’007 (Rousset, 2008).

Two independent methods were used to identify putative loci under selection, as their inclusion in analyses can have an impact on the conclusions drawn. First, coalescent-based simulation methods (Beaumont and Nichols, 1996) were performed with the software LOSITAN (Antao *et al.*, 2008) with samples size equal to the collected samples assuming an island model of 100 islands. A total of 100,000 independent loci were generated with the infinite allele mutation model. Simulated distribution of F_{ST} values conditional to heterozygosity under a neutral model were obtained and thus compared to observed F_{ST} values to identify potential outlier loci. Second, a multinomial Dirichlet model using BAYESCAN v2.01 (Foll and Gaggiotti, 2008) was used to measure the discord between global and population-specific

allele frequencies (based on F_{ST} coefficients). While this method does not take into account the population structure, simulations have shown BAYESCAN to have lower type I and II errors than coalescent-based methods (Narum and Hess, 2011). Log10 values of the posterior odds (PO) > 0.5 and 2.0 were taken as 'substantial' and 'decisive' evidence for selection (Jeffreys, 1961). The false detection rate was set at 0.05 and 0.01, adjusting the log10(PO) significance thresholds corresponding to the 0.5 and 2.0 values considered before correction.

Population differentiation was estimated per pairs of samples and overall using the unbiased F_{ST} estimator (θ of Weir and Cockerham, 1984) and statistical significance assessed using permutation tests implemented in GENEPOP'007 (Rousset, 2008). Two independent approaches were then used to the population structure within the samples; first a Bayesian MCMC clustering approach was implemented in STRUCTURE 2.3.4 (Pritchard *et al.*, 2000). This software clusters all individuals into a predefined number of clusters (*K*) by minimizing overall deviation from HWE and linkage equilibrium within clusters. Considering the likelihood of high levels of gene flow in this highly migratory pelagic species, the admixture model with correlated allele frequencies was used to reflect the most likely pattern of population connectivity. We also allowed the model to include prior information on sampling location (Hubisz *et al.*, 2009). Five independent runs were carried out for each predefined value of *K*, with *K* = 1-4. A burn-in period of 400,000 steps and 600,000 MCMC simulations was used. As STRUCTURE is likely to only detect the highest level of differentiation among the samples (Evanno *et al.*, 2005), a subsequent STRUCTURE analysis was performed on each identified cluster (*K*) containing multiple samples, to identify finer scale population structure within these clusters (Vähä and Primmer, 2006). Additionally, discriminant analysis of principal components (DAPC) (Jombart *et al.*, 2010) was conducted using ADEGENET (Jombart and Ahmed, 2011; Jombart, 2008) implemented in R (R Development Core Team, 2014). This multivariate approach uses synthetic variables to identify differences between groups, while minimising differences within groups. The data was first transformed using Principal Component Analysis (PCA), and to avoid over-fitting the a-score (the difference between the observed discrimination and discrimination based on random groups) was used to determine how many principal components (PC) to retain. Discriminant analysis was then carried out on the spawning populations while the feeding aggregations were then added as supplementary individuals.

2.7 Statistical analyses of the SNPs (Work package 4)

All markers were checked for null alleles and potential genotype scoring errors with MICROCHECKER (Van Oosterhout *et al.*, 2004). LOSITAN (Beaumont and Nichols 1996; Antao et al. 2008) was used to determine whether the loci used were under either directional or balancing selection. Analyses were run with 100,000 simulations under the ''Force mean F_{ST}'', and ''Neutral mean F_{ST}'' alternatives. Confidence interval was 0.95, and the False Discovery Rate was set at 0.1.

Population differentiation was estimated per pairs of samples and overall using the unbiased F_{ST} estimator (θ of Weir and Cockerham, 1984) and statistical significance assessed using permutation tests in GENEPOP'007 (Rousset, 2008). Discriminant Analysis of Principal Components (DAPC) (Jombart *et al.*, 2010) was conducted using ADEGENET (Jombart and Ahmed, 2011; Jombart, 2008) implemented in R (R Development Core Team, 2014). This multivariate approach uses synthetic variables to identify differences between groups, while minimising differences within groups. The data was first transformed using Principal Component Analysis (PCA), and to avoid over-fitting the a-score (the difference between the observed discrimination and discrimination based on random groups) was used to determine how many principal components (PC) to retain. Discriminant analysis was then carried out on the spawning populations while the feeding aggregations were then added as supplementary individuals.

3. RESULTS

3.1 Microsatellite loci (Work package 4)

In total 1,231 fish were successfully genotyped for the 14 microsatellite loci (Table 4; Figure 5).

Table 4. Sample information indicating classification as spawning or feeding, year and location of the sample, and the number (n) of individuals included in the analyses using microsatellite loci.

Sample Name	Type	Acronym	Latitude/Longitude	Year	n
Canada	spawning	CAN	48.2; -64.8	2011	165
USA	spawning	USA	42.6; -70.8	2012	98
Ireland	spawning	IRE	52.9; -11.9	2012	188
France	spawning	FRA - FRA01*	45.0; -2.3	2012	204
		FRA02*	45.7; -1.4		
		FRA03*	47.0; -3.2		
Greenland	feeding	GRE	64.8; -30.7	2011	88
Iceland	feeding	ICE	64.9; -11.1	2010	77
Iceland	feeding	ICE	63.7; -23.6	2011	317
Faroe Islands	feeding	FAR	62.1; -4.6	2011	94

*See Table S1 for pairwise comparisons of samples from France.

The number of alleles per locus was high, ranging from 8 (*Sscom62*) to 47 (*Sscom55*) (Table 5). No null alleles or scoring errors were identified. Departure from HWE was identified in two of the 160 exact tests, neither of which remained significant after Bonferroni correction for multiple tests.

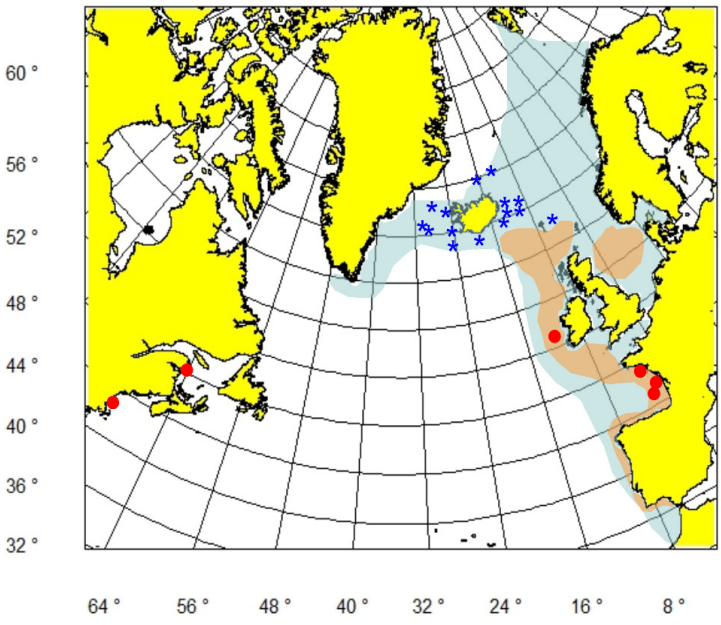

Figure 5. Sampling map showing the location of all samples in the analyses. Spawning samples (red) and feeding aggregations (blue) are indicated, with the extent of the Eastern spawning area shaded orange, and the area covered by the summer feeding migration shaded pale blue (updated until 2015).

Table 5. Genetic diversity of spawning Atlantic mackerel (*Scomber scombrus*) collected at four geographical locations. For each locus the number of alleles (n_a), expected (H_E) and observed (H_O) heterozygosities are shown. Tests that were significant are shown in bold. No tests were significant after correction for multiple tests. CAN: Canada, USA: United States of America, IRE: Ireland, and FRA: France.

Samples		**CAN**	**USA**	**IRE**	**FRA**
Locus	n_a	$H_O(H_E)$	$H_O(H_E)$	$H_O(H_E)$	$H_O(H_E)$
Sscom04	27	0.703(0.757)	0.701(0.740)	0.803(0.814)	0.789(0.800)
Sscom07	10	0.794(0.796)	0.825(0.764)	0.757(0.783)	0.826(0.800)
Sscom08	16	**0.409(0.463)**	0.480(0.446)	0.596(0.660)	0.667(0.668)
Sscom10	11	0.731(0.744)	0.684(0.723)	0.711(0.698)	0.700(0.712)
Sscom25	29	0.732(0.777)	0.714(0.807)	0.888(0.887)	0.873(0.864)
Sscom43	11	0.752(0.718)	0.735(0.754)	0.777(0.764)	0.711(0.740)
Sscom52	13	0.703(0.696)	0.694(0.699)	0.681(0.703)	0.770(0.714)
Sscom55	47	0.939(0.938)	0.948(0.934)	0.926(0.932)	0.926(0.931)
Sscom57	11	0.685(0.694)	0.633(0.669)	0.707(0.669)	0.667(0.699)
Sscom66	14	0.661(0.677)	0.541(0.590)	0.707(0.678)	0.695(0.669)
Sscom50	35	0.915(0.922)	0.918(0.926)	0.952(0.938)	0.868(0.941)
Sscom49	11	**0.497(0.516)**	0.459(0.480)	0.505(0.509)	0.512(0.518)
Sscom48	17	0.848(0.836)	0.840(0.821)	0.786(0.816)	0.788(0.837)
Sscom62	8	0.457(0.493)	0.529(0.498)	0.556(0.606)	0.522(0.598)

No linkage disequilibrium was identified between the 14 loci, and no outlier loci were identified with either BAYESCAN or LOSITAN (Figure 6). All loci were therefore used in all further analyses.

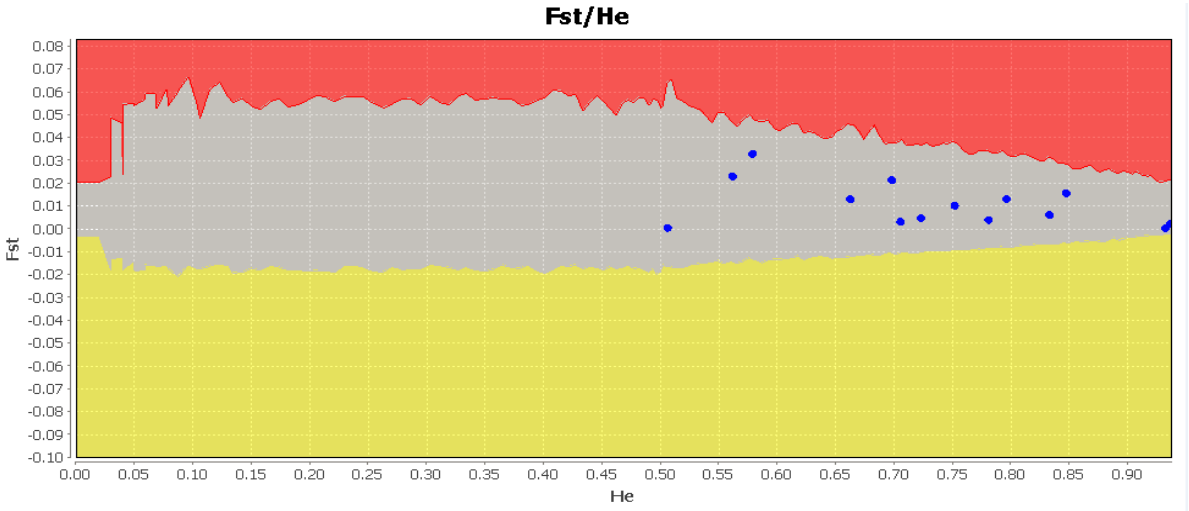

Figure 6. Results of the coalescent-based simulation method for the detection of outlier loci performed in LOSITAN. Blue circles represent each marker, markers within the yellow area are considered to be under balancing selection; those within the red area are considered to be under directional selection; while those in the central grey area are selectively neutral. All markers used during this study are neutral.

Population differentiation was determined for each pair of populations, and the F_{ST} values are given in Table 6. Significant differences were identified between the North-Western and North-Eastern Atlantic samples, but no difference was identified within them (i.e. between Canada and USA; and France and Ireland).

Table 6. Pairwise genetic differentiation (F_{ST} below diagonal) and their associated *p*-values (above diagonal) between samples collected at spawning locations. Significant values are shown in bold. CAN: Canada, USA: United State of America, IRE: Ireland, and FRA: France.

	CAN	FRA	IRE	USA
CAN	-	0.00	0.00	0.26
FRA	**0.01193**	-	0.49	0.00
IRE	**0.01624**	-0.00002	-	0.00
USA	0.00048	**0.01348**	**0.01793**	-

Analyses of the spawning samples with both the Bayesian MCMC approach and DAPC determined that 2 groups were present, STRUCTURE suggested a value of K= 2 as the statistically most likely (ln(K)= -20945.0 ± 17 SD; Table 7a).

Table 7. Hierarchical Bayesian cluster analysis carried out in STRUCTURE (*K,* and associated mean and Standard deviation (St.dev) for LnP(*K*)) using all spawning populations (a) and subsequent detected group analyses using Eastern (b) and Western (c) populations. A total of 5 independent runs were performed for K=1-4 with a 400,000 burn- in and 600,000 iterations. Bold values indicate the most likely number of *K* groups detected in our sample collection.

	LnP(*K*)		**LnP(*K*)**		**LnP(*K*)**	
	a)		b)		c)	
K	Mean	St.dev	Mean	St.dev	Mean	St.dev
1	-21198.0	10.5	**-20101.4**	10.6	**-12553.5**	10.1
2	**-20945.0**	17.0	-20135.6	18.4	-12557.9	14.3
3	-20961.9	18.2	-20165.1	20.3	-12675.5	21.0
4	-20988.7	21.6	-	-	-	-

While all individuals indicated admixture between the clusters, the 2 clusters corresponded with the North-Eastern and North-Western Atlantic groups (Figure 7). Subsequent analysis within these clusters showed no further population differentiation (Table 7b and c).

For the discriminant analysis of principle components, 39 PC and 3 discriminant analyses (DA) were retained. Results were comparable with other analyses, with 2 main groupings identified; North-

Eastern and North-Western Atlantic groups. Greater differences were identified within the North-Western Atlantic than within the North-Eastern Atlantic samples (Figure 8).

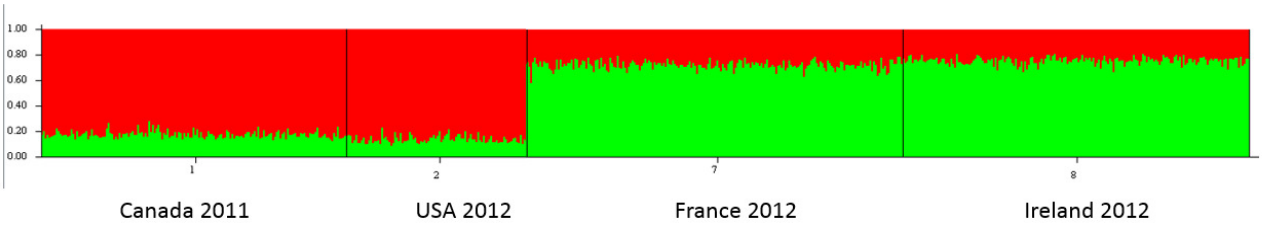

Figure 7. Hierarchical Bayesian cluster analysis carried out in STRUCTURE using all spawning populations for K = 2. Within each plot, each vertical bar represents an individual while colours indicate the different clusters detected. All individuals show admixture, but spawning individuals are clearly differentiated into North-Western (Canada and USA; predominantly red) and North-Eastern (France and Ireland; predominantly green) Atlantic components.

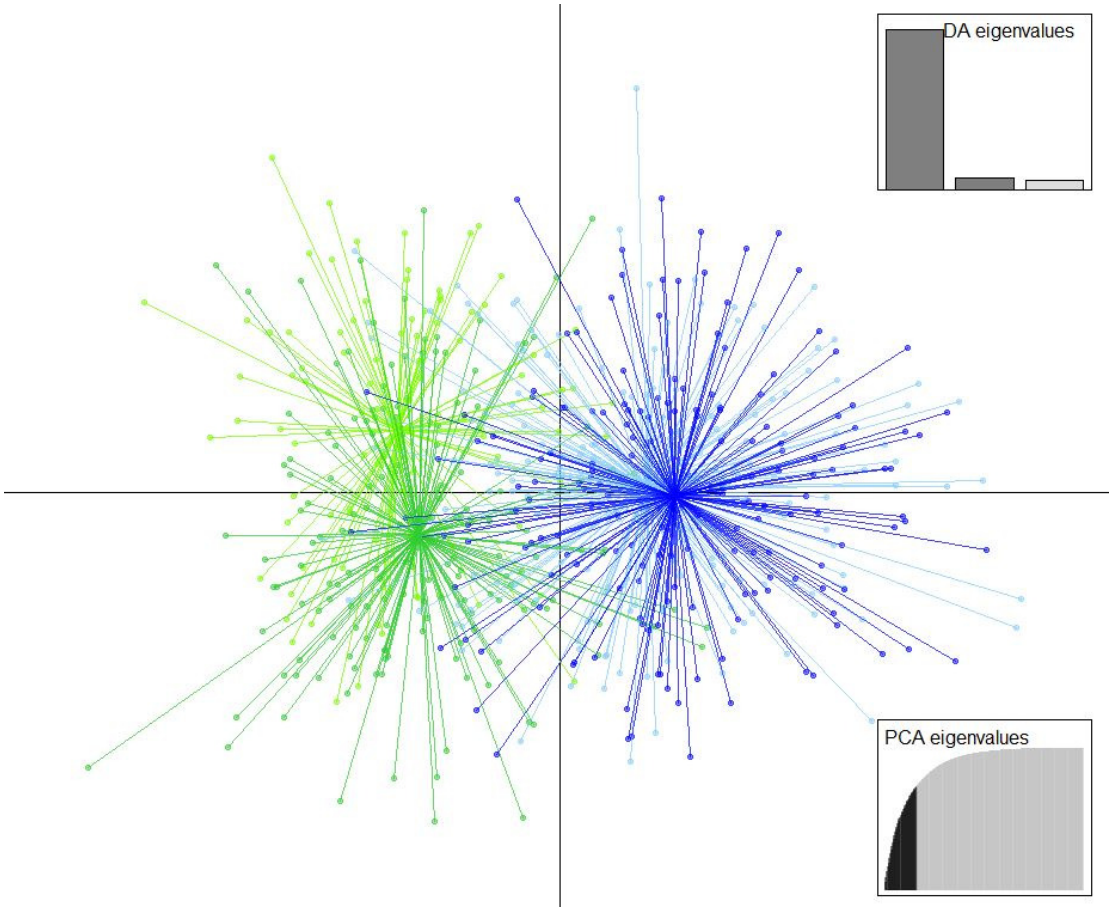

Figure 8. Discriminant analysis of principle components (DAPC) for the spawning samples. The principal component (PC) retained were determined with a-score. Canada (dark green); USA (light green); France (dark blue); Ireland (light blue).

The inclusion of the feeding aggregations in the analyses gives a clear indication of the origin of these migrating fish, with both STRUCTURE (Figure 9) and DAPC (Figure 10a-e) clustering these samples with the European spawning samples.

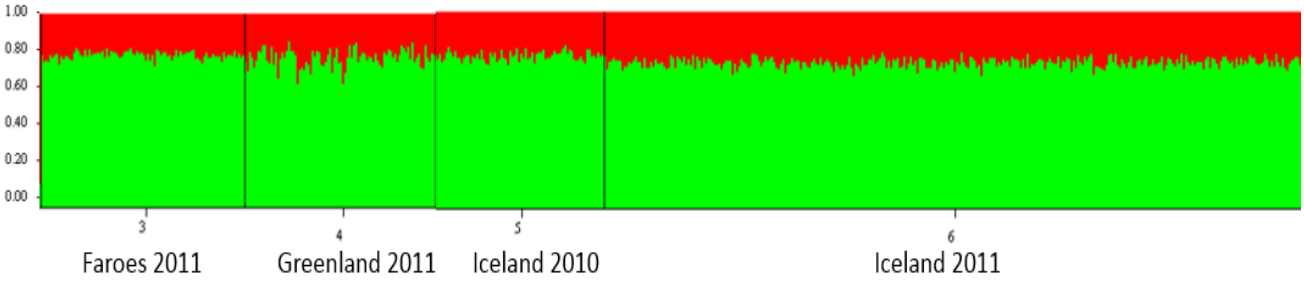

Figure 9. Hierarchical Bayesian cluster analysis carried out in STRUCTURE using all feeding aggregations for K = 2. Within each plot, each vertical bar represents an individual while colours indicate the different clusters detected. Individuals from the feeding aggregations are assigned to the North-Eastern spawning component.

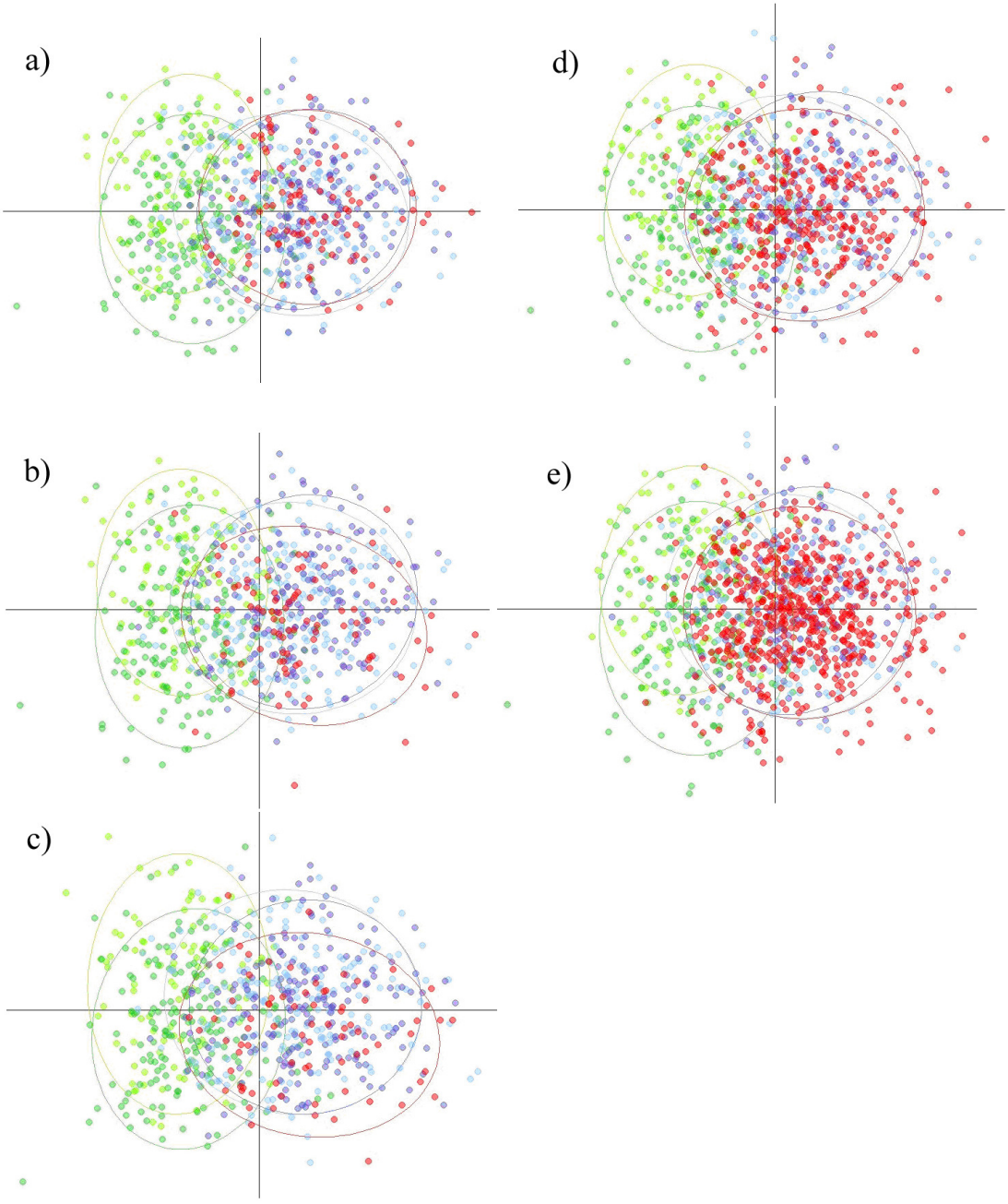

Figure 10. Discriminant analysis of principle components for spawning samples (Canada, dark green; USA, light green; France, dark blue; Ireland, light blue) and feeding aggregations (red): a) Faroes; b) Greenland; c) Iceland 2010; d) Iceland 2011; e) all feeding aggregations combined.

3.3 SNPs sequencing (Work package 4)

The RAD sequencing was highly successful. For the single individual that was sequenced paired end 67,611 contigs were sequenced (a total of 35,466,883 base pairs (bp)), the shortest was 180 bp, the longest was 1,043bp. The median contig length was 575bp, the mode was 720bp and occurred 473 times (Figure 11). Average sequence coverage of contigs was 54.26x; with 306,213 contigs with greater than 5x coverage.

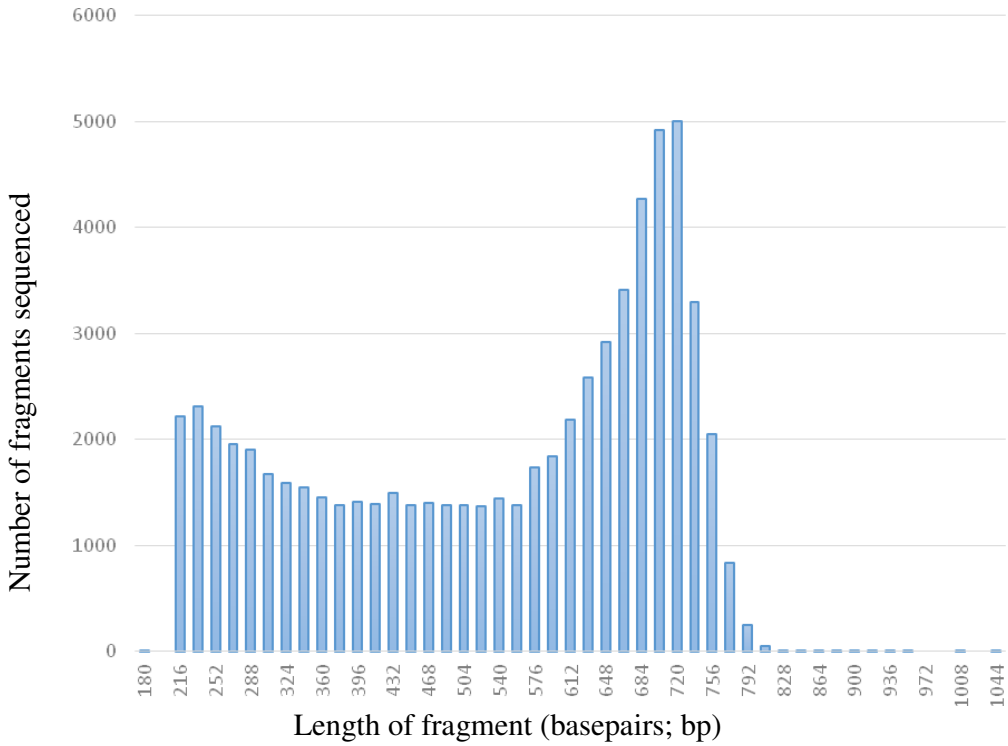

Figure 11. Histogram showing the distribution of fragments size of sequenced contigs for the paired end RAD sequencing.

For the single read sequencing, the mean number of reads per sample was 2,776,015, with a total of 419,178,239 reads. From the 20 individuals sequenced for each of the spawning populations, 8 Canadian and 1 Irish individual did not exceed the 2,000,000 read threshold, and are excluded from subsequent analyses (Figure 12). This yielded 39,503 SNPs; after analysis to remove contigs with low depth, low sequence quality, high numbers of flanking SNPs, 22,008 SNPs remained. Additional data filtering to

remove SNPs with high levels of missing data, poor quality genotypes, and low MAF reduced the dataset to 10,700 SNPs.

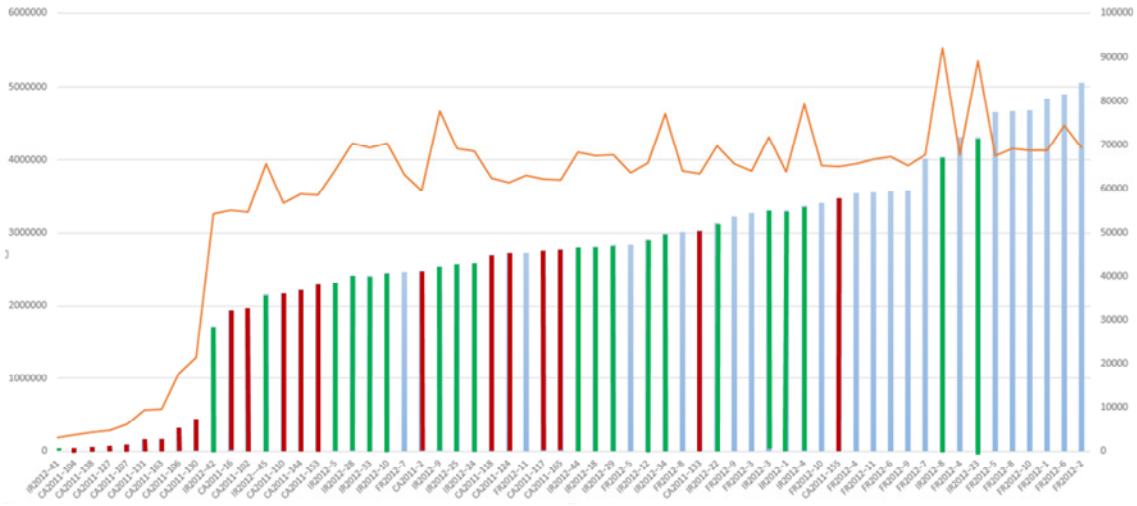

Figure 12. Distribution of the single read sequencing: columns show the number of reads per individual (Canada, red; Ireland, green; France, blue). The orange line shows the number of contigs with a depth of between 5x and 500x.

3.4 SNPs genotyping (Work package 4)

A total of 96 SNPs were genotyped on the Fluidigm Biomark™ for 1,129 individuals. In total 75 loci were successfully transferred from the Illumina sequencing to the Biomark. The 21 loci that failed were due to irregular clustering patterns (which may have been caused by monomorphic loci, paralogues, etc.). A total of 1,079 individuals were genotyped at more than 90% of loci, and were used for further analyses. One further locus was removed from the data set, due to a low number of individuals that were successfully typed at this locus. The remaining 74 loci were all genotyped for more than 90% of individuals. Departure from HWE was identified in 94 of the 296 exact tests within spawning populations considered (Table 8), neither of which remained significant after Bonferroni correction for multiple tests.

Table 8. Genetic diversity of spawning Atlantic mackerel (*Scomber scombrus*) collected at four geographical locations at SNPs loci. For each locus, expected (H_E) and observed (H_O) heterozygosities are shown. Tests that were significant are shown in bold. Significant tests after correction for multiple tests are indicated with a *. CAN: Canada, USA: United States of America, IRE: Ireland, and FRA: France.

	CAN		**USA**		**IRE**		**FRA**	
SNPs loci	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E
SS0004	0.348	0.423	**0.255**	**0.370**	**0.337**	**0.414**	**0.261**	**0.406**
SS0012	0.500	0.375	**0.469**	**0.380**	0.209	0.206	0.185	0.168
SS0015	0.128	0.120	0.098	0.093	0.305	0.301	0.312	0.306
SS0016	**0.042**	**0.117**	0.020	0.019	0.089	0.124	0.130	0.159
SS0017	0.044	0.044	0.128	0.156	0.193	0.210	**0.102**	**0.156**
SS0018	0.204	0.245	**0.059**	**0.161**	0.032	0.071	0.097	0.092
SS0020	0.162	0.149	**0.039**	**0.177**	0.053	0.076	**0.153**	**0.229**
SS0021	0.021	0.062	0.098	0.093	0.110	0.104	0.185	0.168
SS0022	0.108	0.102	**0.098**	**0.192**	0.067	0.089	**0.083**	**0.153**
SS0023	**0.514**	**0.428**	0.471	0.444	**0.432**	**0.382**	0.425	0.404
SS0024	**0.386**	**0.494**	**0.383**	**0.494**	**0.547**	**0.499**	0.500	0.499
SS0026	0.290	0.292	0.426	0.400	0.409	0.385	0.394	0.379
SS0027	0.571	0.500	0.438	0.489	**0.315**	**0.365**	**0.406**	**0.454**
SS0028	**0.245**	**0.453**	0.420	0.442	0.362	0.380	**0.198**	**0.378**
SS0029	0.265	0.259	0.300	0.255	0.129	0.121	0.129	0.121
SS0030	**0.217**	**0.340**	**0.196**	**0.291**	**0.207**	**0.294**	**0.319**	**0.361**
SS0031	0.378	0.368	0.400	0.365	0.355	0.382	0.384	0.385
SS0032	0.449	0.500	**0.327**	**0.475**	0.477	0.494	0.457	0.485
SS0034	**0.568**	**0.477**	**0.490**	**0.407**	0.419	0.412	**0.466**	**0.410**
SS0035	0.303	0.298	0.340	0.375	0.395	0.362	0.319	0.322
SS0036	**0.383**	**0.494**	**0.391**	**0.496**	**0.391**	**0.485**	0.444	0.470
SS0037	0.489	0.442	0.412	0.438	0.363	0.337	0.312	0.278
SS0038	**0.612**	**0.498**	0.490	0.497	**0.234**	**0.359**	**0.391**	**0.466**
SS0039	0.292	0.330	0.373	0.407	0.500	0.492	0.484	0.461
SS0042	0.457	0.415	0.300	0.332	0.217	0.193	0.110	0.104
SS0043	0.532	0.496	**0.583**	**0.486**	**0.474**	**0.420**	0.387	0.403
SS0044	0.313	0.342	0.449	0.440	0.444	0.458	0.398	0.421
SS0046	0.408	0.475	0.400	0.461	**0.394**	**0.329**	0.391	0.396
SS0047	**0.243**	**0.428**	**0.592**	**0.470**	**0.333**	**0.375**	**0.292**	**0.353**
SS0048	0.500	0.500	0.500	0.500	0.493	0.457	**0.324**	**0.452**
SS0049	0.521	0.474	0.412	0.407	0.411	0.420	0.344	0.338
SS0050	**0.327**	**0.408**	0.367	0.390	0.330	0.303	0.370	0.352
SS0051	**0.351**	**0.499**	**0.647**	**0.481**	**0.540**	**0.462**	**0.534**	**0.487**
SS0052	**0.353**	**0.484**	**0.620**	**0.484**	0.237	0.229	0.151	0.184
SS0053	**0.388**	**0.470**	0.460	0.498	**0.418**	**0.490**	**0.437**	**0.500**
SS0054	0.102	0.097	0.118	0.111	**0.495**	**0.420**	**0.323**	**0.383**
SS0055	0.521	0.489	**0.373**	**0.493**	0.404	0.400	0.452	0.421
SS0057	0.487	0.482	0.400	0.471	0.460	0.477	0.444	0.469
SS0058	0.438	0.463	0.529	0.497	**0.537**	**0.495**	**0.409**	**0.483**
SS0059	0.455	0.474	0.510	0.470	**0.333**	**0.387**	0.344	0.375
SS0060	0.458	0.500	0.510	0.468	0.333	0.356	0.333	0.344
SS0061	0.311	0.369	0.375	0.395	**0.284**	**0.326**	0.290	0.278
SS0063	**0.408**	**0.498**	**0.396**	**0.498**	**0.598**	**0.500**	**0.456**	**0.500**
SS0064	0.357	0.398	0.333	0.305	0.316	0.356	0.352	0.317

	CAN		USA		IRE		FRA	
SNPs loci	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E
SS0065	**0.265**	**0.359**	0.319	0.347	**0.347**	**0.402**	0.451	0.459
SS0066	0.354	0.364	0.400	0.385	**0.553**	**0.500**	**0.570**	**0.498**
SS0067	0.429	0.493	**0.306**	**0.470**	**0.372**	**0.478**	**0.433**	**0.499**
SS0068	0.313	0.385	**0.275**	**0.398**	0.495	0.490	**0.385**	**0.500**
SS0069	**0.167**	**0.278**	0.213	0.254	0.392	0.361	0.362	0.348
SS0070	0.438	0.474	0.510	0.484	0.473	0.491	**0.389**	**0.499**
SS0071	0.396	0.385	**0.280**	**0.461**	**0.391**	**0.476**	**0.253**	**0.471**
SS0072	**0.125**	**0.249**	**0.292**	**0.458**	0.449	0.467	**0.291**	**0.458**
SS0073	0.027	0.027	0.059	0.093	**0.107**	**0.211**	**0.029**	**0.082**
SS0076	0.196	0.273	**0.225**	**0.313**	**0.200**	**0.263**	0.239	0.211
SS0077	0.061	0.059	0.020	0.019	0.105	0.100	0.075	0.092
SS0078	0.108	0.102	0.177	0.161	0.173	0.158	0.096	0.116
SS0079	0.163	0.215	0.373	0.407	0.390	0.372	0.391	0.396
SS0081	0.000	0.000	0.059	0.057	0.309	0.275	0.228	0.250
SS0082	0.054	0.053	0.177	0.161	0.080	0.077	0.181	0.164
SS0086	0.184	0.167	0.216	0.251	**0.347**	**0.301**	**0.217**	**0.287**
SS0087	0.000	0.000	0.000	0.000	0.043	0.062	0.000	0.000
SS0089	0.146	0.135	0.078	0.075	0.258	0.256	0.269	0.278
SS0090	0.000	0.040	**0.098**	**0.192**	0.053	0.051	0.183	0.217
SS0091	**0.286**	**0.440**	0.353	0.360	0.158	0.163	0.151	0.139
SS0092	0.449	0.500	0.469	0.497	0.449	0.414	0.391	0.432
SS0093	**0.000**	**0.339**	**0.039**	**0.145**	0.013	0.039	0.014	0.040
SS0094	0.490	0.500	0.440	0.500	**0.527**	**0.481**	**0.411**	**0.478**
SS0095	0.286	0.300	0.200	0.180	0.085	0.082	0.075	0.092
SS0097	0.114	0.108	0.020	0.020	0.056	0.054	0.015	0.015
SS0098	0.163	0.183	0.140	0.196	0.344	0.338	0.413	0.396
SS0099	0.163	0.183	0.353	0.360	0.266	0.303	**0.269**	**0.319**
SS0101	0.143	0.133	0.020	0.019	0.021	0.021	0.054	0.052
SS0107	0.109	0.103	0.078	0.075	0.074	0.090	0.088	0.104
SS0110	0.122	0.150	0.060	0.058	0.075	0.072	0.044	0.044

No linkage disequilibrium was identified between the 74 loci. Five outlier loci were identified with LOSITAN (Figure 13). Five loci (SS0023, SS0042, SS0052, SS0093, SS0107) were indentified as being under directional selection (Table 9). We tested for the potential influence of these loci on the identified genetic structure, by both including and excluding the loci in initial structure analyses (Table 10). However, no difference in population structure was detected when removing the candidate loci, although they are likely to improve the accuracy of assignments, thus they were included in the subsequent analyses.

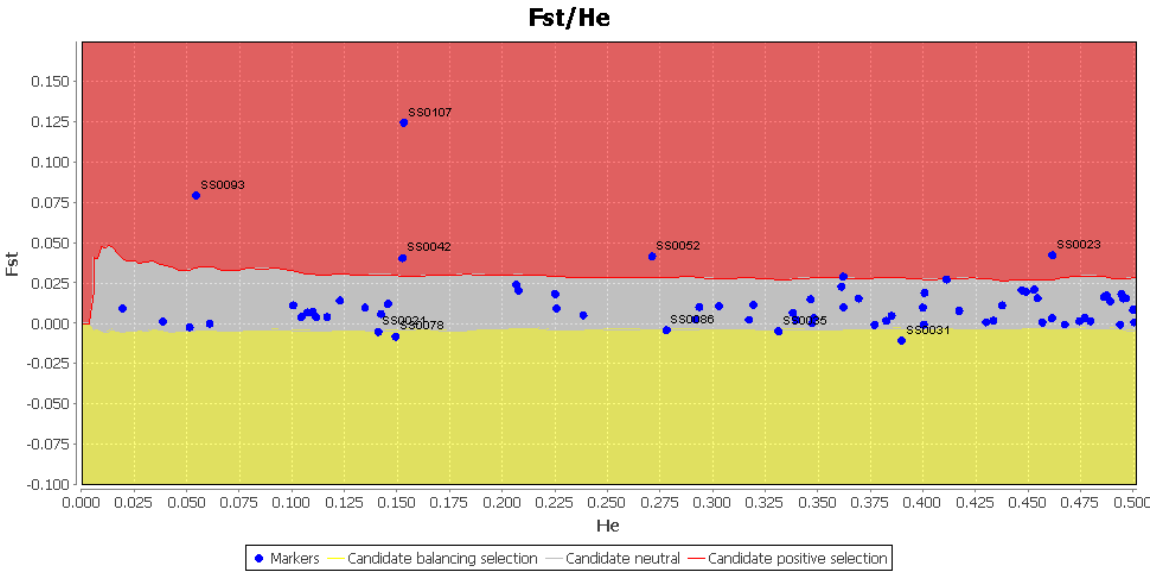

Figure 13. Results of the coalescent-based simulation method for the detection of outlier loci performed in LOSITAN. Blue circles represent each marker, markers within the yellow area are considered to be under balancing selection; those within the red area are considered to be under directional selection; while those in the central grey area are selectively neutral. Five loci were identified as being strongly likely to be under directional selection.

Table 9. Results of the coalescent-based simulation method for the detection of outlier loci performed in LOSITAN. Details for the five loci identified as being strongly likely to be under directional selection are given.

Locus	Heterozygosity	F_{ST}	P(Simul Fst<sample Fst)
SS0023	0.4613	0.042454	0.999294
SS0042	0.152347	0.04074	0.994463
SS0052	0.270978	0.04175	0.998337
SS0093	0.054225	0.079576	0.999936
SS0107	0.153003	0.124795	1

Table 10. Bayesian cluster analysis carried out in STRUCTURE (*K,* and associated mean and Standard deviation (St.dev) for LnP(*K*)) using all spawning populations. A total of 5 independent runs were performed for *K*=1-5 with a 400,000 burn- in and 600,000 iterations. Bold values indicate the most likely number of *K* groups detected in our sample collection. a) all loci included; b) reduced set of loci, with those identified as being under directional selection removed.

	LnP(*K*)		**LnP(*K*)**	
	a)		b)	
K	Mean	St.dev	Mean	St.dev
1	-26095.8	36.1	-25068.1	35.2
2	**-25763.5**	212.1	**-24828.2**	209.2
3	-25848.7	517.0	-24933.8	560.8
4	-26.194.9	1336.7	-25331.2	1458.2
5	-26784.1	2674.7	-25598.6	2059.8

Population differentiation was determined for each pair of populations, and the F_{ST} values are given in Table 11. Significant differences were identified between the North-Western and North-Eastern Atlantic samples, but no difference was identified within them (i.e. between CAN and USA; and FRA and IRE).

Table 11. Pairwise genetic differentiation (F_{ST} below diagonal) and their associated *p*-values (above diagonal) at 74 SNPs loci for the spawning population samples. Significant values are shown in bold, * indicates a highly significant p-value Spawning components: CAN, Canada; USA, United State of America; IRE, Ireland; NS, North Sea; SP, Spain, and FRA: France.

	CAN2011	FRA2012	FRA2013	IRE2012	IRE2013	NS2013	SP2013	USA2012
CAN2011	-	*	*	*	*	*	*	0.198
FRA2012	**0.0466**	-	0.730	0.717	0.955	0.840	0.965	*
FRA2013	**0.0473**	0.0024	-	0.572	0.823	0.606	0.863	*
IRE2012	**0.0576**	0.0024	0.0029	-	0.967	0.282	0.685	*
IRE2013	**0.0425**	-0.0013	-0.0008	0.0003	-	0.524	0.973	*
NS2013	**0.0394**	0.0016	0.0025	0.0050	0.0031	-	0.668	*
SP2013	**0.0398**	0.0011	0.0003	0.0034	-0.0008	0.0030	-	*
USA2012	0.0036	**0.0284**	**0.0323**	**0.0392**	**0.0309**	**0.0205**	**0.0276**	-

Analyses of the spawning samples with both the Bayesian MCMC approach and DAPC determined that 2 major groups were present within the spawning populations. STRUCTURE suggested a value of K= 2 as the statistically most likely (ln(K)= -25763.5 ± 212.1 SD; Table 12a). While all individuals indicated admixture between the clusters, the 2 clusters corresponded with the North-Eastern and North-Western Atlantic groups (Figure 14).

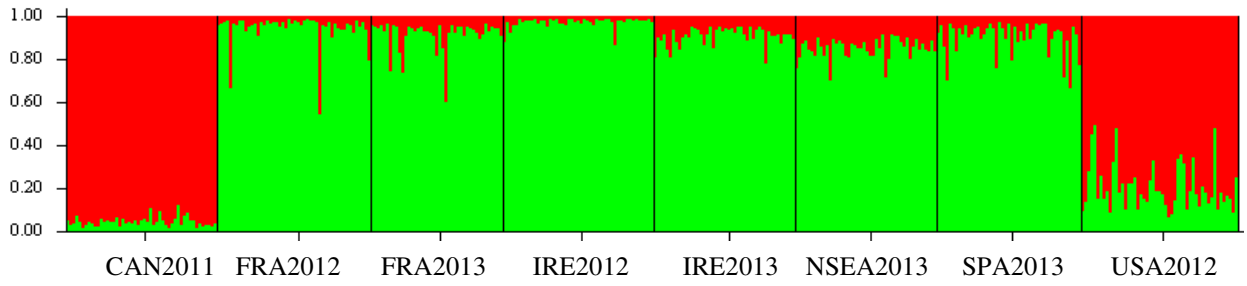

Figure 14. Bayesian cluster analysis carried out in STRUCTURE using all spawning populations for K = 2. Within each plot, each vertical bar represents an individual while colours indicate the different clusters detected. All individuals show admixture, but spawning individuals are clearly differentiated into North-Western (CAN: Canada and USA; predominantly red) and North-Eastern (FRA: France, IRE: Ireland, NSEA: North Sea,, and SPA: Spain; predominantly green) Atlantic components.

Subsequent analysis within these clusters showed no further statistically significant population differentiation (Table 12b and c).

Table 12. Hierarchical Bayesian cluster analysis carried out in STRUCTURE (*K,* and associated mean and Standard deviation (St.dev) for LnP(*K*)) using all spawning populations (a) and subsequent detected group analyses using Eastern (b) and Western (c) populations. A total of 5 independent runs were performed for *K*=1-5 with a 400,000 burn- in and 600,000 iterations. Bold values indicate the most likely number of *K* groups detected in our sample collection.

	LnP(*K*)		**LnP(*K*)**		**LnP(*K*)**	
	a)		b)		c)	
K	Mean	St.dev	Mean	St.dev	Mean	St.dev
1	-26095.8	36.1	**-18885.0**	36.6	**-6861.0**	37.1
2	**-25763.5**	212.1	-19086.9	588.3	-6990.9	416.4
3	-25848.7	517.0	-19459.5	1454.2	-	-
4	-26.194.9	1336.7	-20000.1	2634.8	-	-
5	-26784.1	2674.7	-20120.0	2945.6	-	-

For the discriminant analysis of principle components, 40 PC and 2 discriminant analyses (DA) were retained. Results were comparable with other analyses, with 2 main groupings identified (Figure 15); North-Eastern and North-Western Atlantic groups. Greater differences were identified within the North-Western Atlantic than within the North-Eastern Atlantic samples. Analysis of the European samples indicated that there are subtle differences between spawning areas (Figure 16).

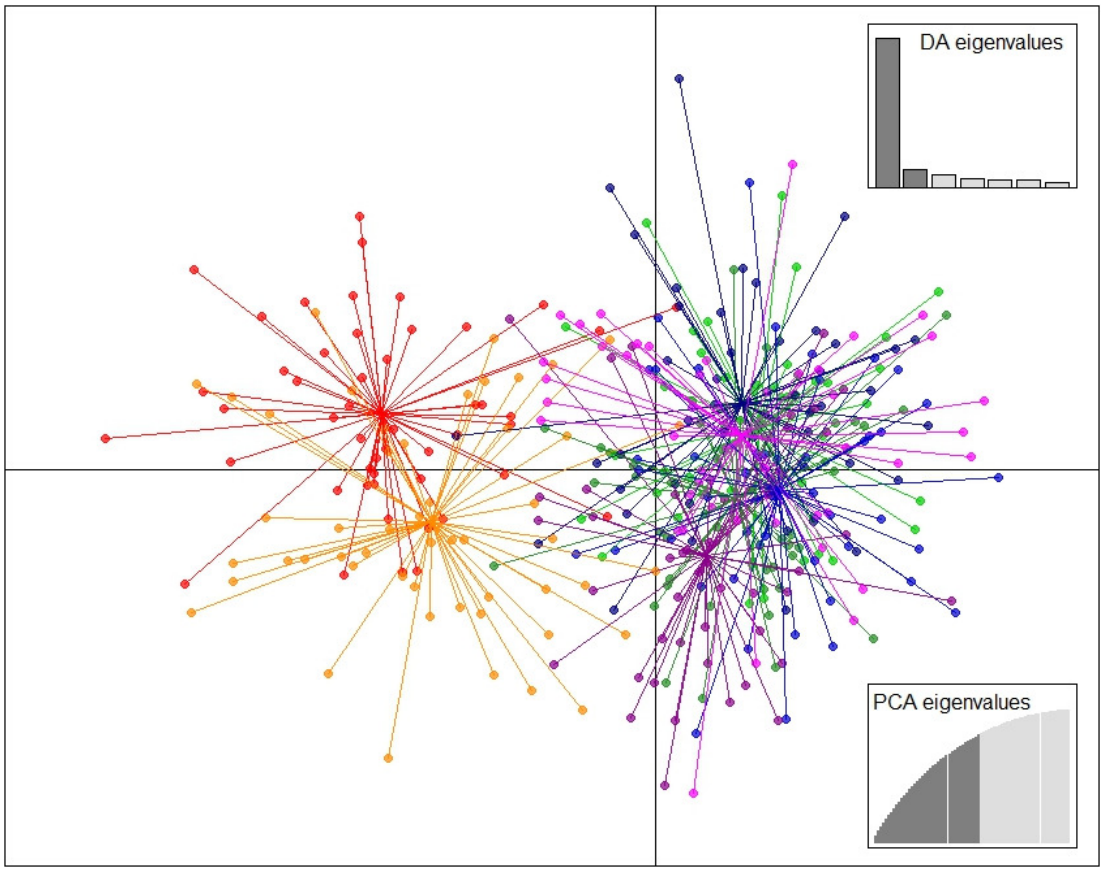

Figure 15. Discriminant analysis of principle components (DAPC) for the spawning samples. The principal components (PC) retained were determined with a-score. Canada (red); USA (orange); France (green); Ireland (blue); Spain (pink); North Sea (purple).

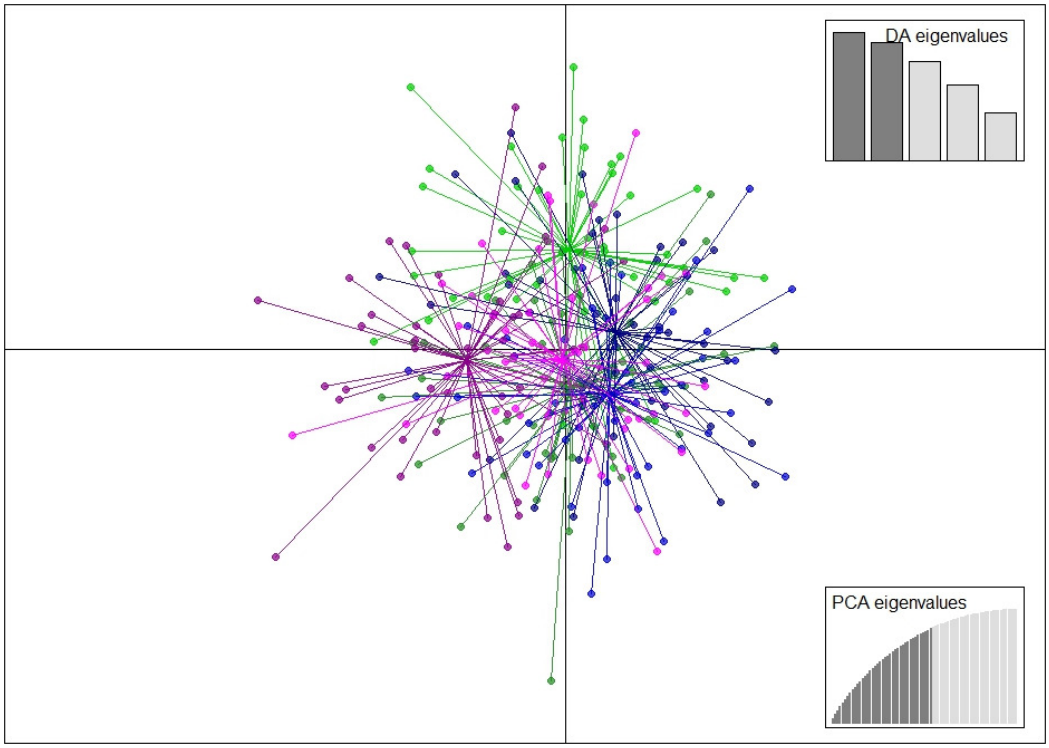

Figure 16. Discriminant analysis of principle components (DAPC) for the European spawning samples. France (green); Ireland (blue); Spain (pink); North Sea (purple).

The inclusion of the feeding aggregations in the analyses gives a clear indication of the origin of these migrating fish, with DAPC (Figure 17) clustering these samples with the European spawning samples.

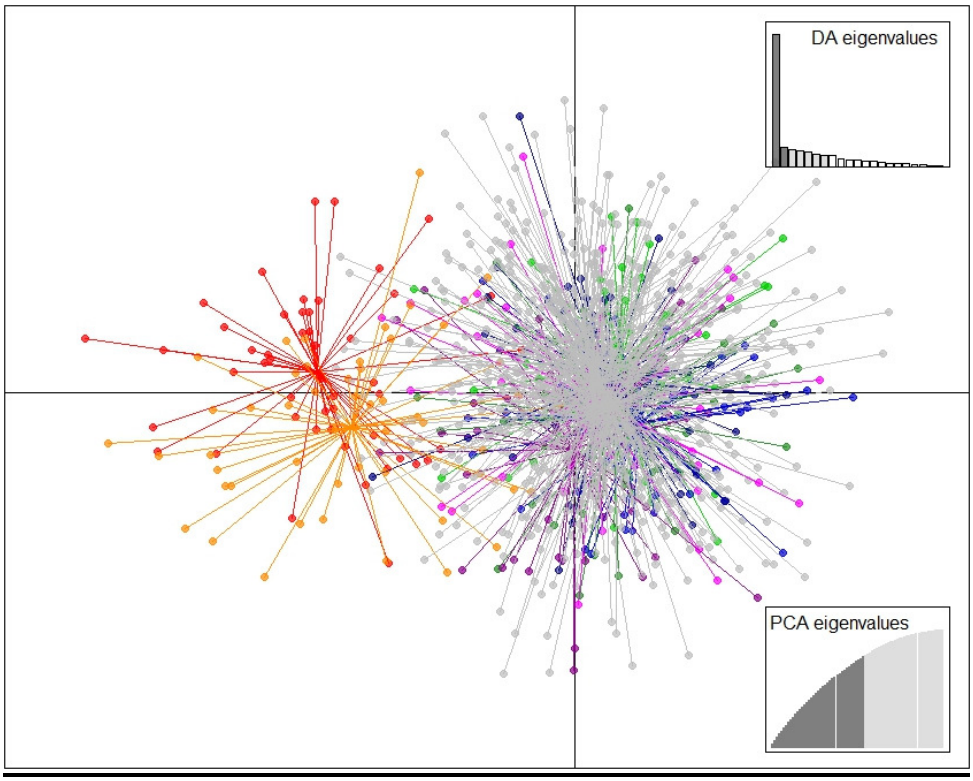

Figure 17. Discriminant analysis of principle components for spawning samples (Canada, red; USA, orange; France, green; Ireland, blue; Spain, pink; North Sea, purple) and all feeding aggregations samples (grey).

4. DISCUSSION

SAM project was developed to answer question related to the changes in the feeding migration of Atlantic mackerel in recent years, i.e. the extension of this species into more northerly and westerly located feeding areas. The main objectives of SAM were:

- To develop new genetic markers using state of the art genome sequencing technique.
- Utilise the resulting genetic markers to analyse samples from different spawning and fishing grounds for mackerel around Iceland, Norway, the Faroe Islands, Canada and adjacent waters for their genetic diversity and stock identification.
- To build a genetic database, including other biological and environmental data for Atlantic Mackerel stocks in the North Atlantic Ocean.

SAM consortium has done a tremendous effort in collecting samples from the distribution range of Atlantic mackerel during spawning and feeding time, from 2010 to 2015. Although we could not genotype all the samples collected during the present study (more than 6,000 samples were collected), samples are now available for future larger projects aiming at understanding the dynamic of the Atlantic mackerel stock(s) and change in the feeding distribution and composition.

SAM has achieved its first objective by successfully developing two types of genetic markers to study the genetic structure and to assess the feeding aggregation composition of the targeted species, e.g. microsatellite loci and SNPs panels.

While microsatellite loci could be easily amplified for all individuals genotyped, the observed level of genetic differences (F_{ST}) between samples collected at spawning grounds was relatively small across the North Atlantic. The main genetic difference was observed between samples collected at the North-western (Canada, USA) and North-eastern Atlantic (France, Ireland). Within each of these components, no genetic differences could be observed.

The genetic differentiation at the developed SNP loci was higher than that observed with microsatellite loci at spawning grounds, although concordant patterns were observed, with the highest difference observed between the North-western and North-eastern Atlantic. However, when within variation was studied in the North-eastern component, some subtle variation could be observed among North Sea, Spain, Ireland and France (Figure 16). Nevertheless, with the samples genotyped, we could not ascertain that this genetic variation was due to genetic structure of populations *per se*, as such subtle genetic variation might as well be due to cohort effects. Currently, SAM consortium is genotyping *circa* 1,000 individuals with SNPs, in addition to the ones already

genotyped, in order to assess why subtle genetic variation was observed among spawning grounds in the North-eastern spawning component.

At present, both developed genetic techniques could clearly distinguish the North-western and the North-eastern spawning components of Atlantic mackerel, and therefore allow the assessment of the origin of mackerel at feeding grounds, e.g. whether mackerel feeding in Icelandic and surrounding waters are of Canadian or European origin. Indeed, individuals collected from the feeding aggregations (Iceland, Faroes, Norway and Greenland) could clearly be assigned to their potential spawning grounds of origin, and both SNPs and microsatellite loci analyses revealed that the Atlantic mackerel in these feeding aggregations were, during the period of investigation, only composed of European Atlantic mackerel. Although not specifically shown within this report, there is much greater potential for Mixed Stock Assessment (MSA) and Individual Assignment (IA) techniques with the SNP loci, as there is clearer differentiation between the stocks, as can be seen by comparing Figures 8 and 15, and also by the differences in the F_{ST} values shown in Tables 6 and 11.
In terms of fisheries, it is now clear that, during the last 8 years, Atlantic mackerel present in Icelandic and Greenlandic waters, around the Faroe Islands and in the Norwegian Sea have originated from European spawning grounds. However, the Atlantic mackerel remains a poorly known species when it comes to its dynamic, biology and its interactions with other species, both as prey and predator. While further studies should be performed on the genetic structure using SNPs (due to the lack of power of microsatellite loci) to understand what sort of genetic variation was captured in the European spawning grounds (cohort effect, different populations, etc…), many more aspects of mackerel biology needs to be investigated in the future.

5. REPORTING AND DISSEMINATION (Work package 5)

SAM dissemination plans were as follows:

The results of SAM were proposed to be a tool for the fishing industry to identify distinct stock units of mackerel in the catch which will enhance sustainable fishing and improve management of mackerel fisheries in the Northeast Atlantic Ocean. Furthermore, it was proposed to be a solution to the on-going debate regarding the allocation of quota of Atlantic mackerel.

The results and outputs were proposed to be published in peer-reviewed international journals. The results were also supposed to be presented at fishery related conferences, to the industry and other relevant stakeholders.

- The results can be used for fisheries management of Atlantic mackerel and will be highly relevant for the negotiations among nations on the division and estimation of fishing stock.
- Other scientists can use the results in other life history studies.
- Other scientists can use the results in stock structure studies on Atlantic mackerel.
- Stakeholders can use the results for more practical and sustainable fisheries.

In addition to provide a new tool for fisheries management, the results were also expected to contribute to new knowledge in the scientific field as limited research is available on the biology and genetic stock structure of mackerel and its recent changes in distribution range. These results were proposed to be presented at fishery related conferences, to the industry and other relevant stakeholders, and will also be published in peer-reviewed international journals.

In response to these statements, SAM progress has been constantly advertised in newspapers or website, nationally and internationally. On the next page, some examples are given.

Icelandic newspaper (2012):

Arfgerð makríls rannsökuð til að varpa ljósi á breytta útbreiðslu í Norður-Atlantshafi:

Leita svara um uppruna í arfgerð makríls

RANNSÓKNIR Vísindamenn Hafrannsóknastofnunarinnar, Matís og Háskóla Íslands ásamt norskum, færeyskum, grænlenskum og kanadískum vísindamönnum rannsaka arfgerð makríls í Norður-Atlantshafi í samvinnu við útgerðarfyrirtæki. Markmiðið er að varpa ljósi á uppruna makríls innan íslenskrar, færeyskrar og norskrar lögsögu, segir á heimasíðu Hafró.

Um er að ræða þverfaglegt verkefni sem byggir á söfnun makrílsýna á mismunandi svæðum og tímabilum og úrvinnslu þeirra með tilliti til DNA-arfgerða, líffræðilegra upplýsinga, vinnslueiginleika ásamt umhverfisgögnum. Jafnframt er stefnt á að greina stofngerð makríls í Norður-Atlantshafi, eða fjölda stofneininga og hvort blöndun eigi sér stað milli ólíkra stofna á veiðislóð, bæði innan Evrópu sem og milli Evrópu og N-Ameríku.

Markmiðið er að verkefnið afli mikilvægra vísindagagna sem geta varpað frekara ljósi á breytingar á útbreiðslumynstri makríls í Norður-Atlantshafi.

Í verkefninu er byrjað á að þróa DNA-erfðamörk fyrir makríl. DNA-erfðamörkin eru notuð sem tæki til stofn- og uppruna-greininga í þeim tilgangi að stuðla að sjálfbærum veiðum og hjálpa til við að spá fyrir um breytingar á útbreiðslu makríls í framtíðinni.

- shá

MAKRÍLL Verkefnið getur stuðlað að sjálfbærum veiðum. FRÉTTABLAÐIÐ/ANTON

Website of the University if Faroe Islands (2012):

Granskingarstuðul til makrelverkætlan

Náttúruvísindadeildin (NVD) á Fróðskaparsetri Føroya hevur fingið játtað granskingarstuðul frá Granskingarráðnum. Fæið er kærkomið og NVD er takksamt fyri stuðulin.

05.06.2012 | Hóraldur Joensen, Náttúruvísindadeildin

Granskingarstuðul
Náttúruvísindadeildin (NVD) á Fróðskaparseturi Føroya hevur fingið játtað 600.000 Dkr í granskingarstuðuli til makrel- verkætlan frá Granskingarráðnum. Krónurnar eru kærkomnar og NVD er takksamt fyri stuðulin.

Tvey sløg av makreli, at síggja til, veidd í íslendskum sjógvi

Stór verkætlan
Trý-ára langa verkætlanin við heitinum: "Stock structure of the Atlantic Mackerel (*Scomber scombrus* L.). An ecological time scale approach to solve stock(s) management" kostar góðar 6 miliónir Dkr. Luttakararnir í verkætlanini eru úr Íslandi, Føroyum, Noregi, Canada og Írlandi.
Granskingarstovnar, fróðskaparsetur og fiskivinna eru umboðað.

Náttúruvísindi og politikkur
Verkætlanin hevur millum annað til endamáls at finna út av stovnauppbýtinum av makreli í Norður-Atlantshavinum eins og veita fakliga grundaða ráðgeving um, hvussu hetta marina tilfeingið kann umsitast burðardygt. Eisini verður miðað ímóti at loysa stóra politiska trætumálið um býti og gagnnýtslu av makrelinum í Norður-Atlantshavinum skynsamt. Harafturat er ætlanin at nýta vunnu vitanina til at spáa um, hvussu makrelurin fer at ferðast í næstu framtíð orsakað av mannaelvdum og náttúrligum veðurbroytingum.

Atknýtt tíðindi

Vitrunnin sátt og semja í makrelmáli

SAM results have also been presented in the following conference:

ICES 2013

Pampoulie C., Óskarsson G., Ólafsdóttir G., Skírnisdóttir S., Ólafsson K., Jacobsen J. A., Joensen H., Siegstad H., Olsen A., Sigurðsson S., Guðmundsson P., Grégoire F., Curti K., Dahle G., Slotte A., Helyar S., and Daníelsdóttir A. K., 2013. Journey of the Atlantic Mackerel into Icelandic waters: Can genetics tell us about its origin? ICES CM/N01, presented at the ICES conference in Harpa in September 2013, Reykjavík, Iceland.

POLSHIFT 2015

Helyar S. J., Óskarsson G., Ólafsdóttir G., Skírnisdóttir S., Ólafsson K., Daníelsdóttir A. K., Jacobsen J. A., Joensen H., Siegstad H., Jansen T., Olsen A., Sigurðsson S., Guðmundsson P., Grégoire F., Curti K., Dahle G., Slotte A., and Pampoulie C., 2015. Genetic structure and population assignment in Atlantic mackerel. POLSHIFT conference 14th-15th of April 2015, Reykjavík, Iceland.

Poster at the ICES 2015:

Helyar S. J., Óskarsson G., Ólafsdóttir G., Ólafsson K., Skírnisdóttir S., Jacobsen J. A., Joensen H., Dahle G., Slotte A., Jansen T., Siegstad H., Daníelsdóttir A. K., Guðmundsson P., Sigurðsson S., Olsen A., Castonguay M., Curti K., O'Hea B., Masse J., and Pampoulie C., 2015. Atlantic mackerel: determining the origins of the feeding migrations and applications to management. (**see attached PDF document**).

SAM results are being published in international journal:

Olafsdottir G., Olafsson K., Skirnisdottir S., Oskarsson G. J., Kohlbach D., Franklinsdottir H., Klitgaard Kvaavik C. E., Morneau R., Chevrier A., Pampoulie C., Helyar S., and Danielsdottir A. K., 2013. Isolation and characterisation of thirty microsatellite loci for Atlantic mackerel (*Scomber scombrus* L.). *Conservation Genetic Resources* 5, 491-494.

6. BUDGET report

Costs for three years 2012-2014	1-MRI	2-MATIS	3-HUG	4-SVN	5-FAMRI	6-UFI	7-FH	8-IMR	9-GINR	10-DFO	TOTAL
Personal cost incl.overhead	716.000	2.058.350	54.000	54.000	252.000	144.000	54.000	690.000	335.840	95.000	4.453.190
Sampling	75.540	23.414	20.047	25.000	278.000	-	27.880	75.193	18.000	25.000	568.074
MS development and screening	-	222.225	-	-	-	-	-	-	-	-	222.225
SNPs identification and screening	-	468.241	-	-	-	-	-	-	-	-	468.241
Meetings-Travel costs	52.000	53.114	18.000	13.047	18.000	30.040	18.000	36.000	36.000	-	274.201
Workshops	60.000		-	-	20.173	-	-	60.000	-	-	140.173
Scholarships	-	207.795	-	-	-	-	-	200.000	-	-	407.795
Project management	104.000	-	-	-	-	-	-	-	-	-	104.000
Total costs	1.007.540	3.033.139	92.047	92.047	568.173	174.040	99.880	1.061.193	389.840	120.000	6.637.899
Applied amount NORA	**291.000**	**477.000**	**43.000**	**43.000**	**178.000**	**18.000**	**43.000**	**371.000**	**36.000**	**-**	**1.500.000**

Financing	1-MRI	2-MATIS	3-HUG	4-SVN	5-FAMRI	6-UFI	7-FH	8-IMR	9-GINR	10-DFO	TOTAL
Own contribution	620.743	1.567.855	54.000	54.000	123.520	101.120	54.000	720.000	360.000	120.000	3.775.238
Other financing (VSR 2012)	135.257	541.028		-	-	-	-	-	-	-	676.285
Other financing (Rannís)		107.961									107.961
Other financing (MII)		50.095									50.095
Other financing (Ice.Industry)		78.225									78.225
Other financing (VÞS)		50.095									50.095
Other Financing FRF (600,000 DKK)	31.040	104.213	6.880	6.880	318.987	61.920	6.880	59.360	3.840	-	600.000
Distributed amount NORA	**220.500**	**533.667**	**31.167**	**31.167**	**125.666**	**11.000**	**39.000**	**281.833**	**26.000**	**-**	**1.300.000**
Total financing	**1.007.540**	**3.033.139**	**92.047**	**92.047**	**568.173**	**174.040**	**99.880**	**1.061.193**	**389.840**	**120.000**	**6.637.899**

7. ACKNOWLEDGMENTS

SAM consortium would like to thank all the cruise members who contributed to the sampling and the collection of data necessary to perform its work. The project was funded by the Icelandic Fisheries Research Fund (Verkefnasjóður sjávarútvegsins), the Nordic Atlantic Cooperation (NORA), the Fisheries Research Fund of the Faroe Islands, and the Icelandic Student Innovation Fund (Rannís) and we would like to thank them all. We would also like to thank Kelle Moreau from the Institute for Agricultural and Fisheries Research, Animal Sciences – Fisheries, Belgium for obtaining samples from the North Sea spawning unit, Kiersten Curti at the National Oceanic and Atmospheric Administration, USA (the Northeast Fisheries Science Center) for obtaining samples from USA (the western Atlantic mackerel stock), and Jacques Masse from IFREMER, France for obtaining samples from France.

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