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Discriminating populations of Atlantic herring mixing in the Norwegian Sea feeding ground using single nucleotide polymorphisms (SNPs)

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ABSTRACT: Atlantic herring *Clupea harengus* feeding in the Norwegian Sea are assumed to consist of Norwegian spring spawners (NSSH), Icelandic summer spawners (ISSH) and North Sea autumn spawners (NSAH). Putative Norwegian autumn spawners (NASH), Faroese autumn (FASH) and spring (FSSH) spawners also feed in the area. However, until there is a method to discriminate between populations in mixed samples, fishery and survey data from the Norwegian Sea will be solely attributed to the predominating NSSH, ultimately causing biased stock assessments. Hence, we evaluated if a panel of 120 single nucleotide polymorphisms (SNPs) associated with spawning characteristics and salinity preferences would be an effective discrimination tool. The overall observed levels of genetic differentiation were high (F_{ST} = 0.57, p < 0.001, 95% CI: 0.51–0.62). Spawners from stocks under current management (NSSH, NSAH and ISSH) were well separated, but the putative populations were not. Discriminant analysis of principal component as well as Structure runs confirmed the differentiation observed with F_{ST}. When the SNP panels were tested on commercial fishery samples of NSSH east of Iceland, up to 16% were assigned to ISSH. This implies that catch data are seriously biased and demonstrates the potential of SNP panels as a tool to solve the problem. However, work is needed to develop improved SNP panels that effectively separate the putative populations from the managed stocks. We recommend that such a tool should be established in regular sampling of fishery and surveys in the Norwegian Sea and accounted for in future stock assessments, advice and management.

KEY WORDS: *Clupea harengus* · Norwegian Sea · Single nucleotide polymorphisms · SNPs · Assignment · Populations · Mixed-stock fisheries · Composition

1. INTRODUCTION

The Atlantic herring *Clupea harengus* Linnaeus, 1758 (Clupeidae) is an important commercial pelagic species that has sustained local communities and the economy of northern European countries for centuries (Smylie 2004). Like many other exploited marine species, the fishery history of herring is marked by its intense exploitation over a long period of time and subsequent drastic concurrent collapses of several of

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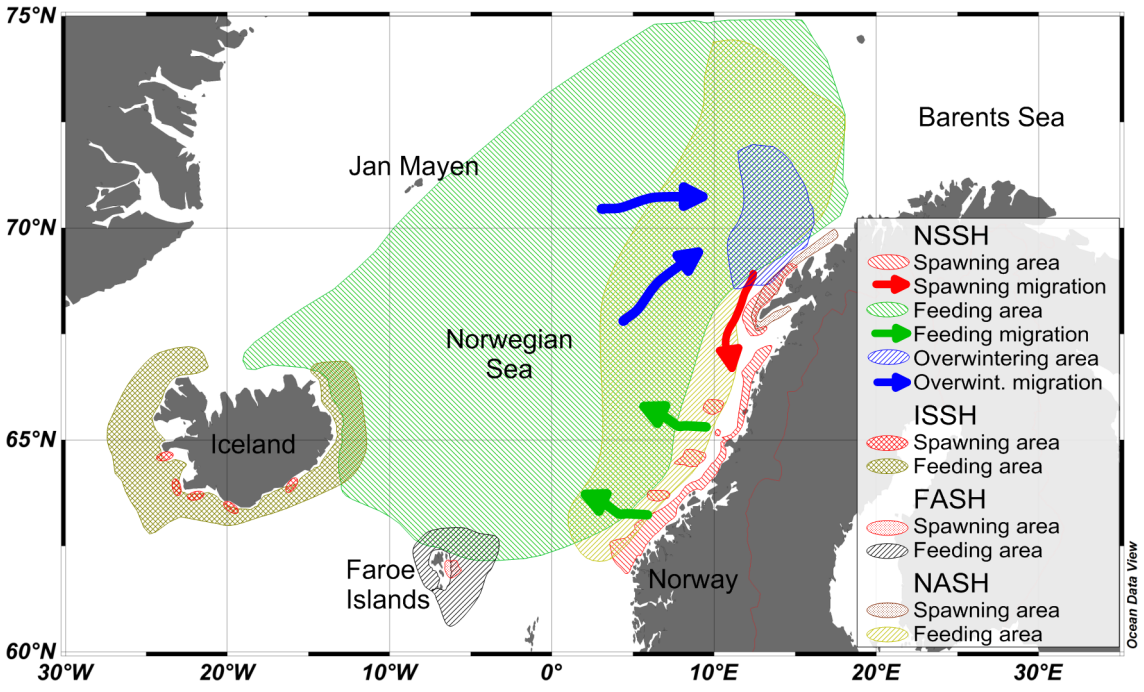

Fig. 1. General migration pattern of Norwegian spring-spawning herring (NSSH) adults and interaction with other surrounding stocks, i.e. Icelandic summer-spawning herring (ISSH), Faroese autumn-spawning herring (FASH), and Norwegian autumn-spawning herring (NASH). Summer feeding grounds of North Sea autumn-spawning herring (NSAH, not shown) reach north to at least 62° N between the Faroe Islands and Norway. Faroese spring-spawning herring (FSSH) is not shown but is found on the Faroe Plateau (same as the FASH area). Redrawn from Pampoulie et al. (2015)

the exploited stocks in the 1960s (Jakobsson 1980, Dickey-Collas
et al. 2010). Worldwide, catches decreased from more than 4 million tonnes to less than a million in a decade (FAO 2022), leading to huge socio-economic challenges in many rural areas including Iceland, coastal areas of Norway and the Faroe Islands (Hamilton et al. 2004, Lorentzen & Hannesson 2006). However, most of the herring stocks surprisingly recovered after depletions over periods of varying length, and are, nowadays, subjected to intense fishing pressure. In the late 1980s, the Norwegian spring-spawning herring (NSSH) stock began to recover, which resulted in a maximum stock level around 2009 of approximately 7.3 million t (ICES 2023a). With increasing stock size, the stock started to feed again in the open oceans between Norway, the Faroe Islands and Iceland in the 1990s (Fig. 1).

According to the current knowledge from biological sampling and macroscopic inspection of gonads, NSSH may mix with 2 other stocks while feeding in the Norwegian Sea (see Fig. 1): Icelandic summer-spawning herring (ISSH) and North Sea autumn spawning herring (NSAH). In addition, there is evidence of Norwegian autumn-spawning (NASH) herring mixing with NSSH both during the feeding and wintering season, but not being managed separately (Husebø et al. 2005). Moreover, the presence of 2 other putative populations, spring-spawning herring (FSSH) and autumn-spawning herring (FASH), has been suggested in Faroese waters, and the total allowable catch for FASH has been set to a level of 12 000 t annually (Faroese Ministry of Fisheries 2023). There is a large-scale international fishery on NSSH in the Norwegian Sea during quarters 2 to 4, with the majority of the catch being taken by Norway, Iceland and the Faroes (ICES 2022, í Homrum et al. 2022). This fishery is reported solely as NSSH stock, although there clearly is a suspicion that it may be a mixed fishery with the other populations. Moreover, the acoustic estimates of 2 international ecosystem surveys which cover the Norwegian Sea during May (IESNS survey) and July to August (IESSNS survey) on an annual basis are only attributed to NSSH. Catch data and survey data form the very basis for stock assessment and advice. If stock composition is not considered in these input data, this could lead to biased stock assessments, increased uncertainty in the quota advice and ultimately reduce managers' ability to maintain sustainable fisheries. There is therefore an urgent need to develop a proper methodology to assess and quantify the level of admixture of herring populations in the Norwegian Sea.

The development of genetic tools to discriminate between herring populations in fisheries and surveys is promising. However, few studies have been performed on the genetic characteristics of herring stocks around the Norwegian Sea basin, and across the entire geographical range of the species in the last decade. A study using 24 presumably neutral microsatellite loci failed to detect any genetic structure among the main spawning component of herring around the Norwegian Sea (Pampoulie et al. 2015). The only detected signal was genetic differences between the Norwegian local spawning populations from different fjords and other spawning grounds around the Norwegian Sea, as well as pronounced genetic differences among these fjord spawning populations (Pampoulie et al. 2015). None of the North Atlantic stocks around the Norwegian Sea, i.e. FASH, FSSH, ISSH and NSSH, could be discriminated. Recent studies based on whole genome sequencing have shown considerable structure among Atlantic herring populations and revealed hundreds of loci associated with ecological adaptation (Martinez Barrio et al. 2016, Lamichhaney et al. 2017, Han et al. 2020). For instance, the genes for thyroid-stimulating hormone receptor (TSHR), the SOX11 transcription factor (SOX11), calmodulin (CALM), and oestrogen receptor 2 (ESR2A), all with a significant role in reproductive biology, were among the loci that showed the most consistent association with spawning time throughout the species range (Lamichhaney et al. 2017). In addition, a large number of loci associated with adaptation to brackish waters have been documented (Lamichhaney et al. 2017, Han et al. 2020). These recent studies based on whole genome sequencing have paved the way for much more powerful genetic discrimination of herring stocks and its potential application to fisheries management using selected single nucleotide polymorphisms (SNPs) associated with the loci controlling ecological adaptation. These studies have also revealed 4 inversions on chromosomes 6, 12, 17 and 23 associated with ecological adaptation, and haplotype divergence among northern and southern populations of herring (Pettersson et al. 2019, Han et al. 2020, Jamsandekar et al. preprint doi:10.1101/2023.10.23.562618). These types of supergenes or haploblocks have often been shown to reflect distinct evolutionary trajectory of populations linked to adaptation to local environmental conditions (Formenti et al. 2022, Matschiner et al. 2022, Theissinger et al. 2023). The information obtained from the evolution of these supergenes can be used to ascertain genetic divergence among locally adapted populations or ecotypes which should be considered for management and conservation (Formenti et al. 2022, Theissinger et al. 2023). Attempts have indeed shown that herring populations can be adapted to very local conditions (Corander et al. 2013, Guo et al. 2016, Kerr et al. 2018, Han et al. 2020). Herring populations that are known to occupy various habitats with differences in temperature and salinity and exhibit differences in spawning time were recently identified as ecotypes using whole-genome data (Han et al. 2020).

The main objective of the present study was to explore how well these recently developed genetic tools discriminate herring populations in the Norwegian Sea feeding area. More specifically our aim was: (1) to use a panel of 120 SNPs associated with spawning characteristics of herring (Lamichhaney et al. 2017) to discriminate the different spawning populations assumed to mix east of Iceland during the feeding season and (2) to assess the actual potential for a better assignment of individual herring to specific spawning stocks in the commercial fishery in the area.

2. MATERIALS AND METHODS

2.1. Collection of samples

Spawning samples of Atlantic herring *Clupea harengus* used during this project were the same as those used in Pampoulie et al. (2015) (see Fig. 2, Table 1). Tissue samples from muscle, gills or fins were sampled in 2 ml tubes containing 96% ethanol. The otoliths of the fish were sampled for age reading. Additional samples were collected during fishery activities on the fishing grounds (filled circles, Fig. 2). A total of 551 individuals were collected at the different spawning locations (see Table 1), while a total of 498 individuals were collected at the fishing grounds during the feeding period (see Fig. 2, Table 1).

2.2. DNA extraction and genotyping

DNA was isolated from either muscle tissue, fin clips or gills using the AGOWA mag Midi DNA Isolation Kit (AGOWA) following the manufacturer's protocol or the HotShot method (Truett et al. 2000). DNA quality and quantity were determined with a NanoDrop Spectrophotometer (Thermo Fisher Scientific). A total of 120 SNPs were genotyped for all individuals (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m739p227_supp.pdf for a description

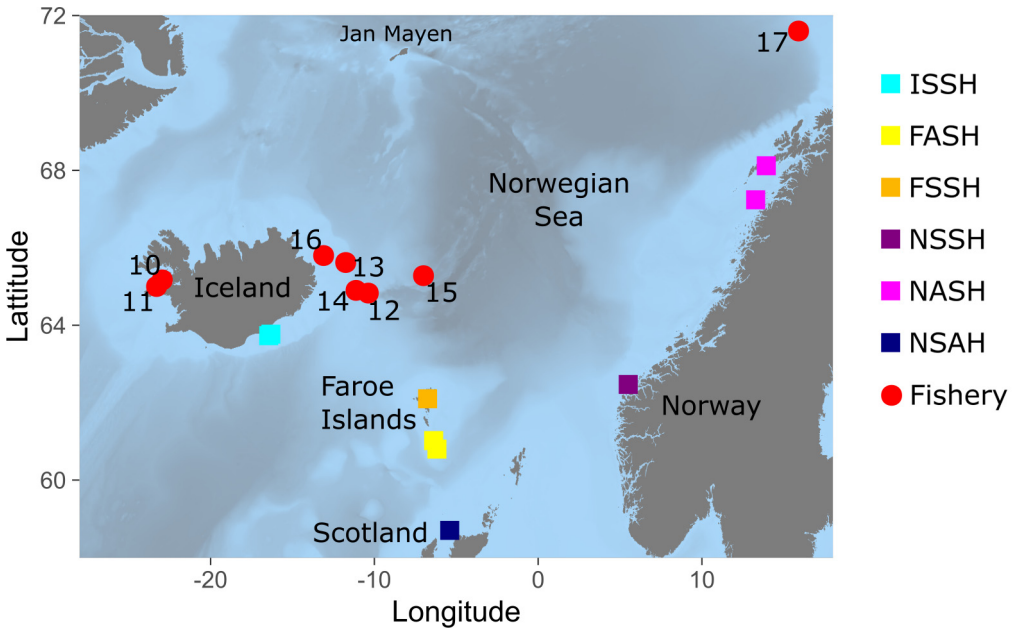

Fig. 2. Locations of tissue samples collected from spawning grounds (squares) and from fisheries on feeding grounds (circles) of the different herring stocks by the Marine and Freshwater Research Institute (MFRI) and the international collaborators. See Table 1 for definitions of population abbreviations. Numbers beside the fisheries circles refer to the station numbers in Table 1

of the SNPs). This SNP panel was previously used to reveal that genetic factors associated with timing of reproduction were shared between genetically distinct and geographically distant populations (Lamichhaney et al. 2017). The chromosomal positions of the 120 SNPs are indicated in Fig. S1 as material for comparison with í Kongsstovu et al. (2022, their Fig. 7).

Standard Biomark protocols were followed (Standard BioTools 2023). Briefly, pre-amplification PCR

Table 1. Samples of Atlantic herring used for (1) the genetic characterization of spawning aggregations and (2) the assignment of individuals from mixed-fishery samples to defined spawning aggregations. For both analyses, a panel of 120 SNPs was used. No.: station number; N: number of individual samples collected at the main and at suspected spawning grounds; S: spawning; F: mixed fishery during feeding

Population	No. and acronym	Sample name	N	Sampling date (mo/yr)	Lat.	Long.	Type
Faroese autumn-spawning herring	1 FASH	FASH_Munkagrunnur	54	11/2009	60.80	−6.18	S
	2	FASH_Landgrunnur	32	11/2009	61.02	−6.38	S
Faroese spring-spawning herring	3 FSSH	FSSH	40	3/2011	62.10	−6.75	S
Icelandic summer-spawning herring	4 ISSH	ISSH411	93	7/2009	63.73	−16.45	S
	5	ISSH473	94	7/2010	63.77	−16.32	S
North Sea autumn-spawning herring	6 NSAH	NSAH (Scotland)	46	9/2010	58.70	−5.40	S
Norwegian spring-spawning herring	7 NSSH	NSSH2012	94	2/2012	62.47	5.50	S
Norwegian autumn-spawning herring	8 NASH	NASH Lofoten2	50	8/2010	67.24	13.28	S
	9	NASH Lofoten1	48	8/2010	68.12	13.93	S
Icelandic summer-spawning herring	10 ISSH	Sild2011-142	48	11/2011	65.17	−22.95	F
	11	Sild-2011-stöð5	81	1/2011	65.00	−23.30	F
Norwegian spring-spawning herring	12 NSSH	Sild-2011-120	46	8/2011	64.83	−10.37	F
	13	Sild-2011-121	42	9/2011	65.62	−11.75	F
	14	Sild-2018-68	43	10/2018	64.90	−11.12	F
	15	Sild-2018-69	49	10/2018	65.28	−7.00	F
	16	SILD2010-149	38	9/2010	65.80	−13.10	F
	17	SILD2010-150	99	10/2010	71.60	15.90	F

reactions were carried out; in this step all the forward (specific target amplification, STA) and reverse primers (locus specific primer, LSP) for the panel of 96 SNPs are 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 is carried out in 5 µl volumes (1.25 µl of genomic DNA, 2.5 µl of 2× Multiplex PCR Master Mix [Qiagen], 0.5 µl of 10× primer 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 s, 60°C for 4 min), and post-PCR, the product (PreAmp DNA) was diluted 1:100 with dH_2O prior to genotyping.

Multiplex SNP genotyping was conducted using SNP Type Genotyping Assays in Fluidigm® 96.96 Dynamic Arrays using standard methods (Standard BioTools 2023). Each array was loaded with 94 samples, 1 negative control (H_2O) and 1 positive control (a DNA mix to aid with the identification of heterozygotes). The PCR on the 96.96 dynamic array was carried out in nl volumes, with 5 µl of each of the 96 SNP Type Assays 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, 2× 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 2× Fast Probe Master Mix [Fluidigm], 0.25 µl 20× SNP Type sample loading reagent [Fluidigm], 0.083 µl 60× SNP Type reagent [Fluidigm], 0.03 µl 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 a BioMark HD System (Fluidigm) for PCR cycling according to the manufacturer's instructions. After amplification, the Dynamic Arrays were read on a BioMark HD and scored using the Fluidigm® SNP Genotyping Analysis software.

2.3. Statistical analyses

Samples were analysed for 120 SNPs, after checking for variation and missing data using adegenet 2.1.8 in R (Jombart 2008, Jombart & Ahmed 2011). Markers that had no or very low variation, or more than 10% missing data were excluded from further analyses. Indices of genetic diversity including observed and expected heterozygosity were calculated in adegenet in R (Jombart 2008). Analysis of linkage disequilibrium for all spawning samples and each spawning sample were done using the index $\bar{r}_d$ that accounts for the number of loci sampled as implemented in the function pair.ia() over all pairs of loci in poppr 2.9.3 in R (Kamvar et al. 2014). Hardy-Weinberg equilibrium (HWE) was tested for in all spawning samples and for each spawning sample using the hw.test() function in the pegas library in R (Paradis 2010) over 1000 Monte Carlo replicates.

Global F_{ST} and F_{IS} over all spawning populations and their confidence interval and significance (CI) were estimated in the diveRsity 1.9.90 library in R with 1000 bootstraps over loci (Keenan et al. 2013). Level of differentiation among populations (F_{ST} values), their CI and significance were estimated in the StAMPP 1.6.3 library in R (Pembleton et al. 2013) using the function stamppFst() with 100 bootstraps over loci. Pairwise F_{ST} fixation indexes were calculated across each locus based on allele frequency and the level of heterozygosity according to Weir & Cockerham (1984), in which statistics were adjusted for unbalanced sample size.

Two independent approaches were then used to determine the population structure within the spawning samples. First, a Bayesian cluster analysis approach was performed as implemented in Structure 2.3.4 (Pritchard et al. 2000). This software groups 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 migratory pelagic species, the admixture model with correlated allele frequencies was used to reflect the most likely pattern of population connectivity. Five independent runs were performed for each K, with $K = 1$ to 6. A burn-in period of 100 000 steps and 100 000 Markov chain Monte Carlo (MCMC) simulations were used. Discriminant analysis of principal components (DAPC) (Jombart et al. 2010) was conducted using adegenet (Jombart 2008) implemented in R. Rather than considering global diversity (as a traditional principal components analysis would), this multivariate approach uses synthetic variables to maximize differences between groups, while minimizing variation within groups (Jombart et al. 2010). DAPC relies on data transformation using principal components analysis (PCA) as a first step before discriminant analysis (DA), which makes the variables that are submitted to DA perfectly uncorrelated (Jombart et al. 2010). The over-fitting of the model was avoided by using cross validation implemented by the function xvalDapc() in adegenet. Cross validation provides an objective way to decide how many axes to retain: different numbers are tried and the quality of the corresponding DAPC is assessed by cross- validation. The

number of PCs associated with the lowest mean squared error is then retained in the DAPC. DAPC analysis was carried out on the spawning populations only and then on the combined spawning and feeding aggregations dataset.

The genetic mixture analysis software ONCOR (Kalinowski et al. 2008) was used for the analysis of the feeding aggregation samples. First, we used the leave-one-out test to evaluate how well fish in the reference collection (spawning samples) can be assigned to their population of origin. Secondly, to evaluate assessment accuracy, we used the 100% fisheries simulation option in ONCOR, in which fisheries samples are simulated based on the same samples. We used the same sample size as in the baseline to simulate mixture genotypes with 1000 bootstraps using the method of Anderson et al. (2008). Thirdly, to assess mixing proportions of all the feeding aggregation samples, the mixture analysis was performed using the baseline (spawning groups) genetic data to estimate stock composition of a sample from mixed stock fisheries over 100 bootstraps.

3. RESULTS

3.1. SNPs

A total of 120 SNPs were genotyped on the Fluidigm Biomark for 1049 individuals. Combining spawning and fishing ground samples, a total of 996 individuals were genotyped at more than 90% of loci and were used for further analyses. Significant departure from HWE was identified in several of the SNPs within the samples collected (Table S1, Fig. S2). The index of pairwise association between loci calculated as $\bar{r}_d$ for all spawning samples and for each sample was relatively high since all SNPs were selected due to their potential association with spawning characteristics and salinity preferences (Fig. S3).

3.2. Characterization of the spawning components

When considering the spawning grounds samples, the overall genetic estimates revealed a highly significant F_{ST} (0.46, $p < 0.001$, 95% CI: 0.38–0.50) and F_{IS} (0.22, $p < 0.001$, 95% CI: 0.14–0.36). All pairwise F_{ST} comparisons were highly significant except the ISSH–FASH comparison. However, the level of differentiation was very low among samples collected in Iceland (ISSH) and the Faroe Islands (FASH and FSSH, Table 2) and they are therefore referred to as the ISSH group herein.

When the DAPC was performed, a total of 26 PCs and 2 discriminant factors (DA eigenvalues) were retained. Overall, the results agreed with the observed F_{ST} values, and revealed the presence of 3 main groups: ISSH group (ISSH, FASH and FSSH), NSAH and NSSH (Fig. 3a). NASH exhibited genetic characteristics which were similar to both ISSH and NSSH, and was therefore an intermediate group. An additional DAPC was performed after removing the most divergent groups, i.e. NSAH and NSSH; here 46 PCs and 2 discriminant factors were retained. No additional structure was found between ISSH, FASH and FSSH (Fig. 3b).

The hierarchical Bayesian cluster analysis conducted in Structure highly supported the DAPC results and suggested that the most likely number of

Table 2. Pairwise F_{ST} value (below diagonal) and p-value (above diagonal) between samples collected from spawning locations. Significant values are shown in **bold**. See Table 1 for definitions of population abbreviations

Population	FASH	FSSH	ISSH	NSAH	NASH	NSSH
FASH	–	<0.001	0.170	<0.001	<0.001	<0.001
FSSH	**0.008** (0.004–0.011)	–	<0.001	<0.001	<0.001	<0.001
ISSH	0.002 (–0.001–0.006)	**0.019** (0.014–0.025)	–	<0.001	<0.001	<0.001
NSAH	**0.504**) (0.402–0.583	**0.446** (0.359–0.514)	**0.519** (0.410–0.610)	–	<0.001	<0.001
NASH	**0.188** (0.162–0.207)	**0.111** (0.096–0.127)	**0.225** (0.199–0.249)	**0.408** (0.359–0.453)	–	<0.001
NSSH	**0.696** (0.635–0.742)	**0.634**) (0.578–0.686	**0.714** (0.663–0.755)	**0.749** (0.712–0.785)	**0.339** (0.298–0.374)	–

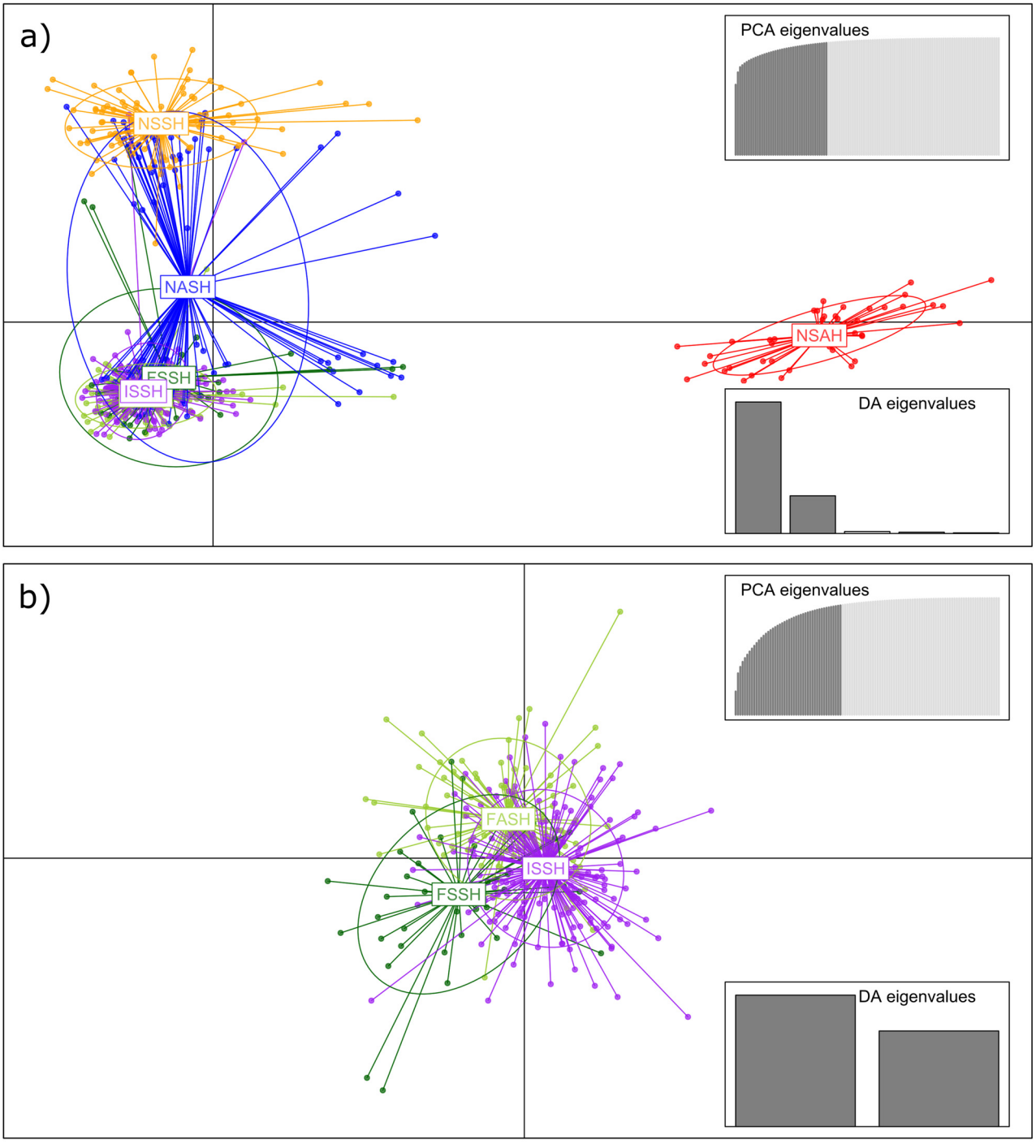

Fig. 3. Discriminant analysis of principal components (DAPC) for (a) all spawning samples and (b) the spawning samples around Iceland (FASH, FSSH and ISSH). The number of PCs retained was determined by α-scores in adegenet. Each circle represents an individual, with the centroid denoting the mean of the population. See Table 1 for definitions of population abbreviations

populations contained in the collected samples was 3 (K = 3, Table 3, Fig. 4). The 3 different groups detected were the ISSH group, NSAH and NSSH (Fig. 4). NASH also exhibited an intermediate characteristic between the ISSH group and NSSH. Indeed, half of the individuals collected as NASH exhibited the genetic signature of the ISSH group and the other half that of NSSH.

ONCOR leave-one-out tests indicated that the spawning individual's assignment to the correct

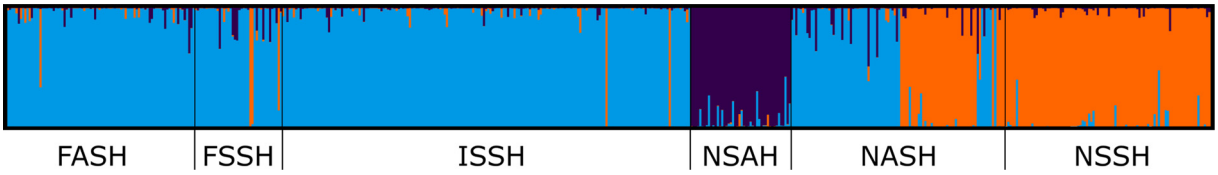

Fig. 4. Bayesian cluster analysis performed in Structure using spawning samples for K = 3. Within the plot, each vertical bar represents an individual while colours indicate the different genetic clusters detected. See Table 1 for definitions of population abbreviations

Table 3. Hierarchical Bayesian cluster analysis conducted in Structure showing probability of *K* from 1 to 6 and associated mean and SD for ln p(*K*) using spawning samples. The bold value indicates the most likely number of populations contained in the collected samples

K	Mean ln p(*K*)	SD ln p(*K*)
1	−60159.62	0.148
2	−35455.86	22.360
3	**−30277.92**	**6.152**
4	−29283.86	596.848
5	−28201.32	4.481
6	−28054.10	20.530

spawning population reached 100% for NSSH and NSAH, but only 66% for ISSH (Table 4). This low rate for ISSH is due to the difficulty in discriminating these herring from FASH and FSSH, which are also self-assigning at a very low rate (Table 4). Therefore, the cross-validation of the baseline spawning samples was problematic for FASH and FSSH.

3.3. Assessment of mixed-fishery composition

A total of 446 fish were collected from the mixed fisheries located in Icelandic and surrounding waters (Fig. 5). One sample was also collected in northern Norway. The analyses of the mixed fishery samples were performed using geographical clusters of mixed fisheries samples (west, northeast and east of Iceland as well as Norway). Due to its mixed composition, NASH was not included in the ONCOR analysis of mixed fisheries samples.

The inclusion of the mixed fishery samples in the analyses gave a clear indication of the origin of these migrating fish with both methods used, i.e. the DAPC (Fig. 5) and ONCOR (Fig. 6, Table 5). First, both analyses showed that 100% of the fish collected at the feeding aggregation located west of Iceland originated from the ISSH group (Fig. 6, Table 5). In the Norwegian Sea area, the samples collected along the east coast of Iceland were composed of mixed origin, with a total of 0 to 16% of the collected fish originating from the ISSH group. These results were again supported by both methods. Finally, roughly 1% of the fish in the fishing ground samples collected north of Norway were assigned to the ISSH group (1 individual). None of the fish originated from the North Sea spawning population (NSAH, Fig. 6). The analysis performed with only ISSH (excluding FASH and FSSH), NASH and NSSH as potential sources of population of origin for the mixed fishery samples led to similar assignment values (Table S2).

Table 4. Proportion of spawning individuals correctly assigned to their population of origin, highest misidentification using the leave-one-out test and 100% simulation in which fisheries samples are simulated based on spawning sample characteristics. The analysis was conducted in ONCOR. N: number of individuals of each spawning sample considered; Correct: percentage of individuals correctly assigned to their population of origin; Avg.: average; SD: standard deviation; 95% CI: 95% confidence interval. See Table 1 for definitions of population abbreviations

	Leave-one-out test			100% simulation		
Spawning population	N	Correct (%)	Largest misidentification	Avg.	SD	95% CI
FASH	68	35.3	50.0% ISSH	0.399	0.06	0.288–0.523
FSSH	33	12.1	39.4% FASH	0.867	0.033	0.799–0.921
ISSH	124	66.1	28.2% FASH	0.786	0.067	0.67–0.902
NSAH	38	100		1.0	0.0	1.0–1.0
NASH	52	0	38.5% ISSH	1.0	0.0	1.0–1.0
NSSH	43	100		1.0	0.0	1.0–1.0

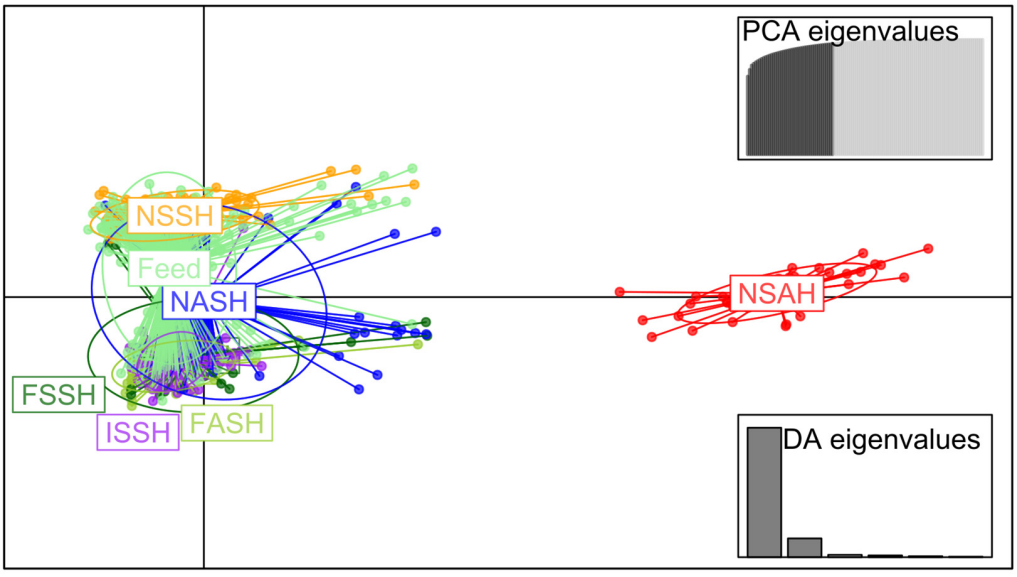

Fig. 5. Discriminant analysis of principal components (DAPC) for spawning samples and mixed-fishery samples. Feed: mixed fishery samples. See Table 1 for definitions of population abbreviations. Note that FASH and FSSH are obscured by ISSH

4. DISCUSSION

4.1. Global genetic structure among spawning grounds

One of the main objectives of the present study was to evaluate the effectiveness of recently developed genetic methods to distinguish among spawning stocks of herring and to determine the composition of the mixed fisheries occurring in the southern Norwegian Sea (notably east of Iceland). There is a need for a better characterization of the populations/stocks regularly occurring in this geographical region, i.e. Icelandic summer spawning (ISSH), Faroese autumn spawning (FASH), Faroese spring spawning (FSSH),

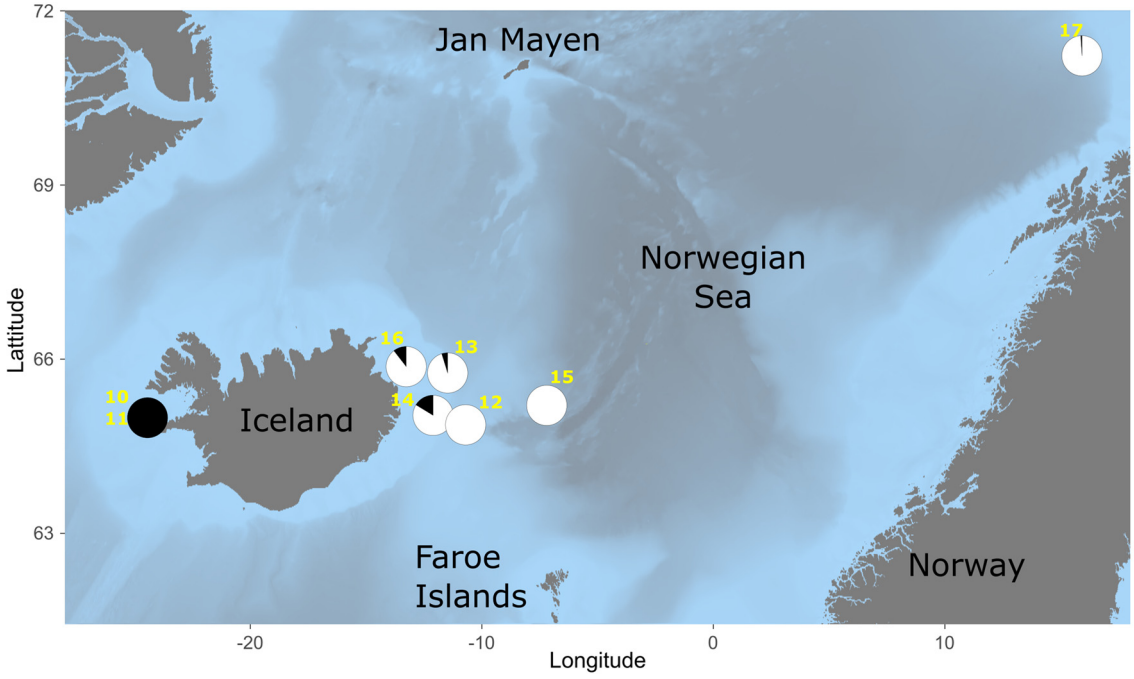

Fig. 6. Composition of the mixed fisheries occurring on the east coast of Iceland and in the Norwegian Sea limits. (Black) ISSH group (ISSH, FASH and FSSH); (White) NSSH. Numbers beside the pie charts refer to the station numbers in Table 1. Removing FASH and FSSH from the ISSH group does not change the results. See Table 1 for definitions of population abbreviations

Table 5. Mixture analysis of fisheries samples performed in ONCOR. Samples collected from the supposed mixed fisheries were tested against 3 spawning components: NSSH, ISSH group (ISSH, FASH and FSSH) and NSAH genetic clusters. No individuals from mixed fisheries were assigned to NSAH by this approach, which is therefore not reported. Area refers to the station numbers reported in Fig. 6 and Table 1. See Table 1 for definitions of population abbreviations

Area	Reporting group	Estimate	95% CI
10–11	ISSH	1.000	1.000–1.000
	NSSH	0.000	0.000–0.000
12	ISSH	0.000	0.000–0.000
	NSSH	1.000	1.000–1.000
13	ISSH	0.048	0.000–0.119
	NSSH	0.952	0.881–1.000
14	ISSH	0.163	0.069–0.256
	NSSH	0.837	0.744–0.930
15	ISSH	0.000	0.000–0.000
	NSSH	1.000	1.000–1.000
16	ISSH	0.105	0.026–0.211
	NSSH	0.895	0.790–0.974
17	ISSH	0.010	0.000–0.030
	NSSH	0.990	0.970–1.000

North Sea Autumn spawner (NSAH) and Norwegian spring spawning herring (NSSH). First, contrary to the microsatellite loci (Pampoulie et al. 2015), the panel of 120 SNPs used during the present study revealed that all the spawning samples were significantly different from each other, except FASH and ISSH (see Table 2). We can therefore confirm that this panel can discriminate the 2 largest herring stocks from this region, ISSH and NSSH, as previously suggested (í Kongsstovu et al. 2022). However, while the differentiation between ISSH vs. NSSH and ISSH vs. NSAH were high (F_{ST} > 0.5), the level of differentiation between ISSH and the Faroese spawning samples was low (F_{ST} = 0.01). In addition, FASH was not genetically different from ISSH, contrary to what has been previously described (í Kongsstovu et al. 2022). On the contrary, the FSSH samples collected in April 2011 were genetically different from both FASH and ISSH. However, the observed level of differentiation between these stocks was low for the loci included in this panel and whole genome sequencing should be carried out to ascertain the existence of the suspected stocks around Iceland and the Faroe Islands. The lack of difference between FASH and ISSH, as well as the differences observed between the present study and the one performed by í Kongsstovu et al. (2022), is likely due to the different SNP panel used. First, the panel of SNPs used in the present study was selected based on knowledge accumulated from the northeast and east stocks of herring (mainly NSSH, NSAH and NASH). Unfortunately, the SNP selection process did not include any individuals from the Iceland–Faroe region and might therefore not accurately capture divergence between the stocks in this area. This was indeed reflected in the performed Structure analyses, which were not capable of discriminating samples from the Iceland–Faroe region. On the contrary, the DAPC clearly showed some structuring among FASH, FSSH and ISSH, but not strong enough to separately assign fishery samples to these components. Second, the panel of SNPs used during the present study contains 4 time less loci than the one used by í Kongsstovu et al. (2022), and the positions on the chromosome map were different. While their 457 SNPs were distributed among almost all chromosomes with high presence on chromosomes 6, 8 and 12, our 120 SNPs were mainly distributed in chromosomes 12 and 15, with very few additional ones on chromosomes 6, 7, 8 and 19. Finally, these 2 studies did not use the same herring spawning samples from the Iceland–Faroe regions, which might also affect the results. Hence, due to these observations and due to the low level of genetic divergence among samples from the Iceland–Faroe region, the assignment of the mixed fisheries samples was investigated using 2 different groups of spawning herring, ISSH vs. NSSH (see Section 4.2).

Finally, the observed results for NASH and the level of differentiation between this sample and both NSSH and ISSH was striking. Half of the individuals from the NASH sample could be assigned to ISSH and the other half to NSSH with high accuracy. This result is unlikely to reflect high gene flow over such a long distance considering the well-established life-history traits of both ISSH (Óskarsson et al. 2009) and NASH (Husebø et al. 2005) and weak indications of long-distance migration of ISSH to Norway (Jakobsson 1961). Hence, these results might merely evidence that selection pressures are similar within the habitat of NASH and the ISSH and/or the NSSH groups. In addition, these results might also reveal misclassification of fish based on biological features. Indeed, fish classified as 'NASH' were from 2 samples, collected close to each other in time (11 and 13 August), near the spawning site. The sample clustering with ISSH consisted of newly spent fish, whereas the one clustering more with NSSH was predominated by resting and early maturing fish representative of this spawning population at that time of year. The genetic clustering is therefore likely correct, as half of the NASH

fish were probably misclassified according to maturation staging, hence reflecting the difficulties in classifying fish solely based on this parameter. The panel of SNPs used in the present study could therefore not properly capture differences among these stocks. Whatever the cause behind this result, we could not draw any conclusions with regards to stock structure involving NASH (either genetically close to ISSH or misclassified), and we therefore removed the NASH sample from the mixed fishery samples analysis.

4.2. Assignment of mixed-stock fisheries to spawning grounds

To our knowledge, previous genetic studies on herring have mainly focussed on discriminating spawning components/populations (Bekkevold et al. 2005, Mariani et al. 2005, Ruzzante et al. 2006, Lamichhaney et al. 2012, Corander et al. 2013, Pampoulie et al. 2015, Guo et al. 2016, Martinez Barrio et al. 2016, í Kongsstovu et al. 2022). This is indeed the first analysis of catch samples collected along the east coast of Iceland and in the Norwegian Sea around the Iceland–Faroe region and adjacent waters. On the basis of our research, only 3 populations could be considered as potential source populations, i.e. the group including ISSH, FASH and FSSH, the NSSH (Norwegian) and the NSAH (North Sea), during the assignment of individuals (see Section 4.1). With the inability to adequately differentiate ISSH from the relatively unknown and poorly defined FASH and FSSH stocks using this SNP panel, we refer to those herring as the ISSH group (ICES 2023b). As suspected, samples collected on the western coast of Iceland (see Fig. 6) were assigned to ISSH at a 100% rate. The Atlantic herring occurring in this region have been known to be part of the ISSH stock for many years, although mixing could have occurred with the second spawning stock of Iceland before it collapsed. Indeed, the Icelandic spring spawning herring (ISPH), which could mix with ISSH in some feeding regions, collapsed in the late 1970s and has not recovered (Óskarsson 2018).

Not surprisingly, the analyses of the fisheries samples collected from the east coast of Iceland revealed the presence of a mixture of the ISSH and NSSH groups (Fig. 6). Most of the fish collected from the mixed fisheries on the east coast of Iceland originated from NSSH, while a portion varying from 0 to 16% originated from the ISSH group. The highest proportion of fish originating from the ISSH group was detected in the northeast and southeast parts of the fisheries in Iceland (Fig. 6). The feeding sample collected in Norway was assigned to NSSH at 99%.

In general, there was an acceptable agreement between the assessment of admixture composition of the fisheries using SNPs related to spawning characteristics and salinity tolerances and the phenotypic (maturity stages) assignment of herring to spawning season and hence stocks. The phenotypic estimate of mixing at the fishing ground east of Iceland averages 10 to 15% (ICES 2023a), and the genetic estimate of mixing from the present study was quite similar (4 to 16%). When comparing the individual genetic assignment using our SNP panel to assignment results using the maturity stages, there was a disagreement for 7 out of 414 cases (1.7%). Considering that the genetic assignment is more powerful, 2 individuals (2.1%) collected on the fishing grounds of ISSH west of Iceland were wrongly assigned as spring spawners (NSSH or other) using maturity stages, while they were genetically confirmed as ISSH fish. On the fishing grounds of NSSH east of Iceland during the autumn, 5 individuals (1.6%) were incorrectly assigned using maturity stages. Three were incorrectly assigned as summer/autumn spawners (ISSH) when the SNP assignment resulted in their identification as NSSH, and the reverse occurred for 2 of them. All these 5 mismatches in the fishery for NSSH were from samples collected in August and September, which are known to be the most problematic months for identification of herring 'stocks' origin when using maturity stages. At this time, NSSH gonads are small and at an early developmental stage, while the gonads of the summer spawners are at a resting stage. In October, the separation on basis of maturity stage is easier, and all the herring were indeed assigned correctly to spawning 'stocks'.

4.3. Fisheries implications

Sustainable fishery management can only be achieved if individuals belonging to different stocks can be distinguished, both at spawning and feeding grounds (Andersson et al. 2024). It is now commonly known that fisheries occur on feeding grounds composed of several stocks. In the case of the Atlantic herring, there is a need to develop cost-effective technologies to discriminate the different known stocks at an international level, and to assess their contribution to mixed fisheries occurring at feeding grounds. Indeed, while assessment and advice on fishing opportunities of the Atlantic herring stocks is coordinated through the International Council for the

Exploration of the Sea (ICES), previous genetic studies have been primarily focussed on regional characterization of spawning components (Bekkevold et al. 2005, Mariani et al. 2005, Ruzzante et al. 2006, Lamichhaney et al. 2012, Corander et al. 2013, Pampoulie et al. 2015, Guo et al. 2016, Martinez Barrio et al. 2016, í Kongsstovu et al. 2022). In Icelandic waters and further east in the Norwegian Sea, there are currently 6 known herring stocks (Fig. 1): ISSH and ISPH, which occupy the continental shelf around Iceland; FASH, assumed to be constrained to the Faroes shelf; FSSH, observed only sporadically in the Faroese fjords and in small numbers, and thought to be a remnant of the spring spawning herring that spawned on the eastern banks of the Faroes in the 1950s and 1960s; NSSH, widely distributed across the whole area; NASH, primarily found in coastal and offshore areas in northern Norway; and NSAH, occupying the North Sea and adjacent waters, including the southern part of the Norwegian Sea (east of the Faroes). Three different management units are currently considered for the Norwegian Sea and adjacent waters, i.e. NSSH, ISSH and NSAH, which are assessed by ICES and managed by coastal states or on a national level (ISSH). The ISSH management unit currently includes ISPH, which has collapsed and not recovered (Óskarsson 2018), and the NSSH unit includes NASH and FASH in the southern Norwegian Sea and, in part, local stocks found along the Norwegian coast and fjords in the eastern Norwegian Sea (Johannessen et al. 2009, Silva et al. 2013; Fig. 2). The stock assessments underlying these management units rely on the ability to discriminate individuals and quantify the different stocks in commercial catches and research surveys. Under current practice, the discrimination of these stocks is based on catch location and macroscopical examination of the maturity stage (i.e. macroscopic categorization of gonad development into 8 stages). While this method has been applied for many years, it has 2 fundamental problems. First, in some periods during the year, the maturity stage can be almost identical for different stocks (e.g. ISSH and NSSH in early August). Secondly, allocation of herring at a certain maturity stage to a specific stock is not always straightforward. The panel of 120 SNPs that we used in the present study was powerful enough to clearly distinguish 4 genetically different stocks: ISSH, FSSH, NSAH and NSSH. However, the observed level of genetic differentiation around Iceland and the Faroe Islands was not large enough to properly assign individuals from the mixed fisheries to every stock separately. For example, we could not discriminate FASH from ISSH, and neither could í Kongsstovu et al. (2022) in their assignment, although they were able to discriminate these 2 putative stocks using population structure analysis. The mixed samples obtained in the present study do not represent the actual multinational fishery for herring in the southern Norwegian Sea. Therefore, we cannot provide an unbiased estimate of mixing in this fishery, but rather indicate the magnitude. Our study therefore still lacks power to clearly assess the differences among the stocks located around Iceland and the Faroe Islands, and also lacks power to assign individuals caught in the mixed fisheries to ISSH, FASH and FSSH separately.

4.4. Future perspectives

The value of an SNP panel for stock discrimination is based on how well stocks of interest have been characterized (Andersson et al. 2024). The Atlantic herring is subdivided into many genetically distinct stocks, but these show no or very limited genetic differentiation at selectively neutral loci due to large population sizes and gene flow that leads to minute genetic drift. To ensure high discriminatory power of an SNP panel, whole genome sequencing of the stocks of interest is required so that the most informative SNPs can be included in the panel (Andersson et al. 2024). The panel of SNPs used during the present study was designed using variation only observed among individuals collected from the east Atlantic Ocean (Pettersson et al. 2019). It is therefore likely that potential genome variations among ISSH and the Faroese stocks were not captured, which indeed hampered our ability to properly assign mixed stock fisheries samples to these stocks. The same reasoning can be used to explain the similar genetic characteristics of NASH and ISSH. The fact that there is no reference genome for the ISSH group might have led to the similar genetic characteristics of NASH and ISSH, which are likely due to fish from both of these populations being subject to similar selection pressure at the loci used during our study. A remedy for this problem would be to carry out whole genome sequencing of population samples of Icelandic and Faroese herring to establish allele frequencies at all SNP loci and compare these with the more than 50 previously sequenced population samples across the species distribution (Han et al. 2020). Considering the relatively low level of divergence among herring populations across their distribution, it is likely that a local reference genome for each putative population of origin will be needed to

fully fathom population structure (Thorburn et al. 2023) and assess mixed-fisheries composition. This would allow the development of a larger, but still small, panel of SNPs which might indeed take the variation among all stocks into account. Consequently, this would allow for a better assignment of mixed fisheries samples to their stock of origin, if divergence among ISSH and the Faroese stocks exists. Both ISSH and Faroese herring (FASH, FSSH) have small population sizes compared to their Norwegian counterparts, for example. They might exhibit hitherto undetected genomic variability reflecting their adaptation to local environmental conditions, as has been shown for other herring ecotypes (Han et al. 2020). It is therefore crucial to assess their contribution to mixed fisheries to avoid their extinction and conserve population genetic diversity of Atlantic herring in the region.

Data availability. The data used during the present study have been deposited in the OSF open-access database and are available at: https://osf.io/5ejwh/.

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