











Population structure discovered in juveniles of Greenland halibut (*Reinhardtius hippoglossoides* Walbaum, 1792)

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Understanding the genetic differentiation among populations of most marine fish requires investigating the differences among spawning grounds. However, this can be challenging as spawning grounds for some species are not well known, or spawning fish are difficult to collect. An alternative is to collect juvenile fish in nursery habitats closely associated with potential spawning grounds. Greenland halibut is a deep-dwelling, commercially important species with at least two identified major offshore spawning grounds in the North Atlantic and weak genetic differentiation across the Atlantic. In this study, we sampled juveniles from three sites representing the Davis Strait spawning area in the northwest Atlantic and one site in the northeast Atlantic representing the primary spawning area along the western slope of the Barents Sea. We applied genotype by sequencing and discovered 90 genetic markers that could be used to assess genetic differentiation among the four sites. The northeast and northwest Atlantic showed major genetic differentiation, supporting the existence of the two primary spawning clusters. Additionally, we found genetic differentiation between the three northwest Atlantic samples implying the existence of more than one spawning area in the northwest.

Keywords: Disko Bay, Frobisher Bay, GBS, juvenile habitat, single nucleotide polymorphism, Svalbard.

Introduction

Biological populations are self-sustaining groups of individuals that are genetically differentiated from each other but can still be related through meta-population dynamics (Reiss *et al.*, 2009). For widely dispersed highly mobile species, such as many marine fish, assessing population structure should focus on sampling individuals on or near their spawning grounds during the spawning season (Laikre *et al.*, 2005). This is because populations are likely to exhibit some level of reproductive isolation, if it exists, especially if individuals return to natal areas to spawn (philopatry). Ultimately, this reproductive isolation may translate into genetic and phenotypic differences among spawning components representing true biological populations (Reiss *et al.*, 2009). However, collecting individuals on spawning grounds during spawning periods can be challenging, especially when these areas and periods are not well known or defined (Boje and Hjörleifson, 2000). As an alternative, nursery habitat likely include juveniles from nearby spawning grounds and may serve as proxies for spawning populations (Beck *et al.*, 2001).

Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum, 1792), is a deep-sea pleuronectid flatfish with a North Atlantic and North Pacific distribution that also includes Arctic shelf seas (Vihtakari *et al.*, 2021). This long-lived demersal piscivore inhabits depths from 200 to 2000 m (Boje and

Hareide, 1993; Jørgensen, 1997) and can spend up to 20% of its time feeding in pelagic zones (Albert *et al.*, 2011). Given its role as a piscivore, it is an important link in Northern/Arctic marine ecosystems. However, many aspects of its life cycle and evolutionary history are not entirely understood. In particular, the species' population structure remains unresolved due to various interpretations of previous research (Riget *et al.*, 1992; Knutsen *et al.*, 2007; Pomilla *et al.*, 2008; Roy *et al.*, 2014; Westgaard *et al.*, 2017; Orlova *et al.*, 2019; Carrier *et al.*, 2020; Wojtasik *et al.*, 2021).

In the North Atlantic, Greenland halibut is currently managed as four offshore stocks. The northwest Atlantic (NWA) stock is distributed within Baffin Bay and Davis Strait; the east Canadian stock extends from Labrador to Newfoundland including the Grand Banks and Flemish Cap; the West Nordic stock encompasses waters of East Greenland, Iceland, and the Faroe Islands, and finally, the northeast Arctic stock (NEA) extends from the Norwegian Sea (including Jan Mayen) to Svalbard and the Barents Sea (see Figure 1 in Vihtakari *et al.*, 2022). These four stocks are based primarily on regional management objectives and assumed spawning grounds, but both population genetics and tagging studies challenge this stock structure (Roy *et al.*, 2014; Westgaard *et al.*, 2017; Vihtakari *et al.*, 2022). Reports based on tagging experiments show migration from Svalbard to north of Iceland (Albert

Received: 6 October 2022; Revised: 10 January 2023; Accepted: 11 January 2023

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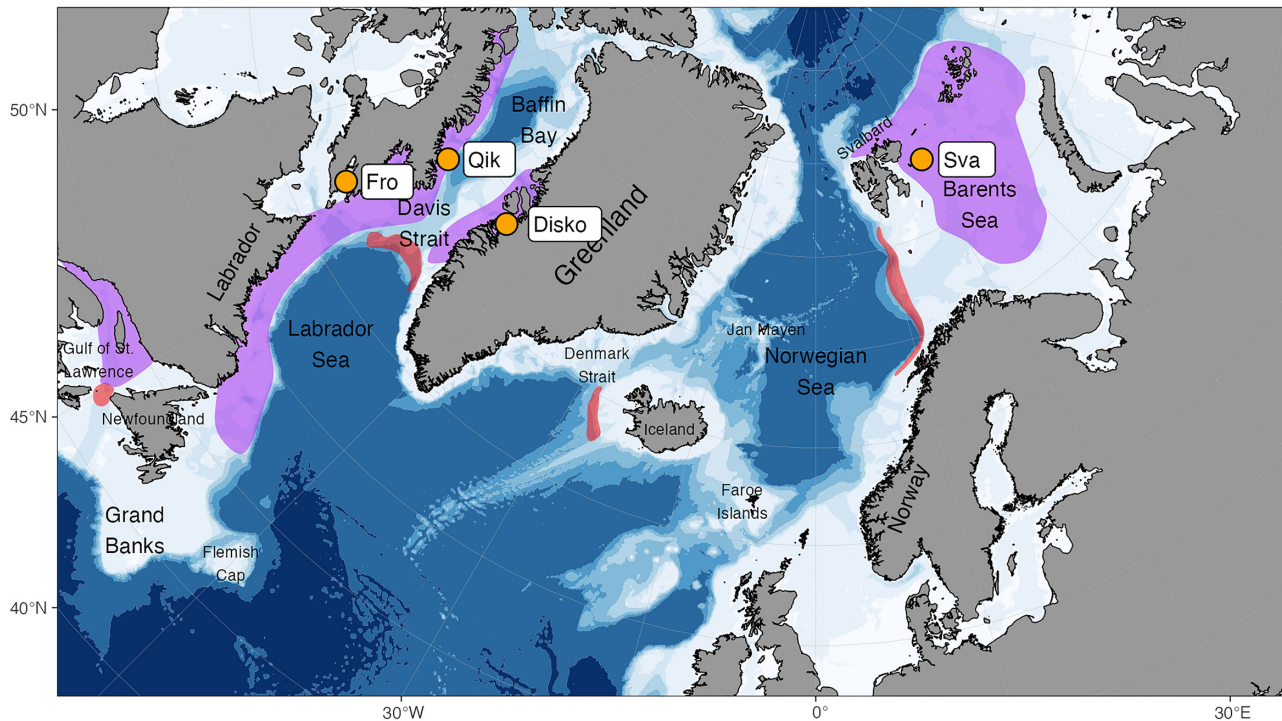


Figure 1. Sampled locations for juveniles in the North Atlantic. Yellow dots indicate the sampling locations (Sva: Svalbard, Disko: Disko Bay, Qik: Qikiqtarjuaq, and Fro: Frobisher Bay). Known spawning grounds (red) and juvenile habitats (purple) are indicated using polygons.

and Vollen, 2015), suggesting connectivity between these two regions. Extensive migration distances to the southwest of Iceland have also been reported, but the number of fish observed showing this migration pattern is small (Vithakari *et al.*, 2022).

Genetic evidence for population structure has been mixed. This is largely due to the nature of the genetic markers used and their likely selection status. Early work showed some structure between the Gulf of St. Lawrence and offshore regions, but this was based on allozyme markers which are derived from expressed enzymes (Fairbairn, 1981). Later work used microsatellites identified in Atlantic halibut (*Hippoglossus hippoglossus*), with some loci showing signs of heterogeneity in Greenland halibut, to support population structure in the NWA, but also in the North Atlantic overall (Knutsen *et al.*, 2007; Pomilla *et al.*, 2008). More recently, studies have used either allozymes, microsatellites from Atlantic halibut, or single nucleotide polymorphisms (SNPs) to suggest possible structure within the western Barents Sea (Wojtasik *et al.*, 2021), the Laptev Sea relative to the Bering Sea (Orlova *et al.*, 2019), or isolation by distance with a porous barrier in the shallow shelf area of Denmark Strait, between the northwest and northeast Atlantic (Westgaard *et al.*, 2017). Genotype-by-sequencing has reaffirmed population structure between the Gulf of St. Lawrence and offshore areas near Newfoundland, but only when loci identified as under selection were included in the assessments (Carrier *et al.*, 2020). Differences between the Gulf of St. Lawrence and offshore stocks have also been linked to differential adaptation to temperature and oxygen (Ferchaud *et al.*, 2022). Studies using strictly neutral markers show that the NWA is a panmictic unit (Roy *et al.*, 2014; Westgaard *et al.*, 2017), however debate continues as to whether this applies to the entire North Atlantic (Knutsen *et al.*, 2007; Roy *et al.*, 2014; Westgaard *et al.*, 2017).

Observations of egg, larvae, and/or spawning females suggest the existence of at least two main spawning areas in the sub-Arctic North Atlantic waters (Figure 1). One consistently considered spawning area is located in the Davis Strait, south of the Canada–Greenland submarine ridge (Jensen, 1935; Gundersen *et al.*, 2010), while another has been associated with the western slope along the Barents Sea (Godø and Haug, 1989; Albert *et al.*, 2001a). In addition, ripe and running fish have been observed in the west of Iceland and southeast of Greenland (Magnusson, 1977; Sigurðsson and Magnusson, 1980), although the associated juvenile nursery habitats are unknown. Other studies suggest additional spawning in the NWA around the Flemish Cap (Junquera and Zamarro, 1992) and in the Gulf of St. Lawrence (Fairbairn, 1981; Carrier *et al.*, 2020). Eggs presumably spawned in Davis Strait are assumed to drift either southward and spread over much of northeast Canadian waters (Wheeland and Morgan, 2020), or northward to populate fjords throughout Baffin Bay including Disko Bay and northwest Greenland fjords. Similarly, in the NEA, offspring spawned along the continental slope recruit to the area around Svalbard, as well as the Barents, Kara, and Laptev seas (Albert *et al.*, 2001a,b; Benzik *et al.*, 2022). Juveniles found in the southwest of Iceland and in East Greenland fjords could also be from related juvenile nursery habitats corresponding to spawning grounds in Icelandic waters.

To assess genetic differentiation between two major spawning areas in the North Atlantic, we collected samples from the main expected juvenile nursery habitat along the slope of the Barents Sea, and three nursery habitats in NWA were presumed to represent the Davis Strait spawning area (Figure 1). We assessed the genetic structure of these samples using SNPs obtained by reference-based variant calling from genotyping-by-sequencing (GBS) libraries. Differences among the collected juveniles would support separate spawning pop-

Table 1. Sampling details for Greenland halibut collected in 2019 on four locations in the North Atlantic. Comment: can the numbers be centered please?

Sample	Acronym	Sampling	Latitude	Longitude	N	Size range (cm)
Svalbard, Norway	Sva	Sept.	78.69°N	30.34°E	76	10.5–38.0
Disko Bay, Greenland	Disko	Nov.–Dec.	68.88°N	–51.52°W	68	18.2–38.0
Frobisher Bay, Canada	Fro	Aug.	62.83°N	–66.58°W	51	7.2–20.1
Qikiqtarjuaq, Canada	Qik	Sept.	68.10°N	–63.78°W	52	18.6–29.8

Average position of each sample is given as decimal degrees.

ulations across the Atlantic, thereby aiding in defining stocks better reflecting the species' biological populations.

Materials and methods

Sampling

In 2019, 279 juvenile Greenland halibut were sampled from juvenile nursery habitats near spawning grounds in northeast and northwest Atlantic (Figure 1). Juveniles were collected from three locations in the NWA representing the Davis Strait spawning site: Disko Bay (Disko), Frobisher Bay (Fro), and Qikiqtarjuaq (Qik), and in the northeast Atlantic from Svalbard (Sva) (Table 1 and Figure 1). The fish were 7–38 cm in length and may have represented ages 0–4 years. Greenland halibut <35 cm were targeted as they are thought to be pre-migratory individuals (Albert and Vollen, 2015; Vih-takari *et al.*, 2022) and therefore representing juveniles seeded from respective spawning grounds. We were able to collect younger juveniles (<20 cm) from Frobisher Bay and Qikiqtarjuaq, Canada, whereas the juvenile fish from Disko Bay, Greenland, and Svalbard, Norway, were larger (but <35 cm). Disko Bay samples were collected from November to December by a commercial trawler. Samples from Frobisher Bay and Qikiqtarjuaq were collected by Fisheries and Oceans Canada inshore trawl surveys conducted during August and September. Samples from Svalbard were collected in September during the annual Barents Sea Ecosystem Survey.

Genotyping, filtering, SNP calling, and selection of loci

Samples were sent to AgResearch Ltd, New Zealand, and sequenced following a GBS procedure. GBS protocol, filtering, SNP-calling, and selection of loci for a reduced number of SNPs for further analysis are available in the Supplementary Information.

Genetic variation and population structure

After selecting 90 SNPs with the largest F_{ST} in LOSITAN, we went back to our original data (163290 SNP) and extracted 37 more individuals for these 90 SNPs that were excluded in the filtering because of more than 10% missing data. Post GBS and filtering resulted in a final two datasets of 5560 ($n = 210$ fish) and 90 SNPs ($n = 247$ fish) used to analyse genetic variation and spatial genetic structure. Preliminary population and locus-specific genetic statistics were assessed with GENEPOP 4.7.0 (Rousset, 2008). Genetic variation within samples was assessed for observed and expected heterozygosities as was sample and locus-specific F_{IS} . Linkage among loci in each sample and over all samples was estimated from 10 000 dememorization steps using 20 batches per run and 5000 iterations per batch. Deviations from Hardy–Weinberg expectations (HWE)

for each sample and locus were estimated using the Hardy–Weinberg probability test with the same number of dememorizations, batches, and iteration as for LD tests. For all multiple tests, correction for false discovery rate was applied using BY method as implemented using the `p.adjust()` function from the stats library in R (Benjamini and Yekutieli, 2001). Genetic differentiation between samples, and their significance, was estimated using theta (Weir and Cockerham, 1984) using GENEPOP.

Discriminant analyses of principal components (DAPC) on clusters pre-defined by sampling location was performed in hierarchy with adegenet 2.1.3 (Jombart, 2008; Jombart and Ahmed, 2011) implemented in R. First, we analysed the data using all four samples and then repeated the analysis using only the three samples from the NWA. We determined the number of principal axes and discriminant factors (DFs) (DA eigenvalues) to retain using the cross-validation approach with 100 repetitions implemented using the `adegenet: xvalDapc()` function.

Population structure was also tested using a Bayesian clustering approach implemented in STRUCTURE 2.3.4 (Pritchard *et al.*, 2000; Hubisz *et al.*, 2009). All runs were performed assuming admixture, correlated allele frequencies, and using the LOCPRIOR option, accounting *a priori* for sampling location. STRUCTURE was run for $K = 1–5$ with five replicates for each K , with a burn-in of 300000 and 1000000 Markov chain Monte Carlo (MCMC) iterations post burn-in. STRUCTURE outputs were analysed in StructureSelector (Li and Liu, 2018). StructureSelector offers a web-based service helping to assess the most likely value of K based on the Evanno method (Evanno *et al.*, 2005). It also produces graphs of the mean $\ln P(K) \pm SD$ across the repeated runs for each K and graphs for MedMed K , MedMean K , MaxMed K , and MaxMean K (Puechmaile, 2016). Finally, CLUMPAK (Kopelman *et al.*, 2015) was used within StructureSelector to produce graphical displays of the results outlining each individual's probability of belonging to a particular recovered genetic cluster.

To further support and visualize genetic differences among locations, we also calculated Nei genetic distances (D_A) (Nei *et al.*, 1983) and constructed a UPGMA tree with 100 bootstraps over loci in Populations 1.2.32 (Oliver Langella, 1999, <http://bioinformatics.org/~tryphon/populations/>). We also analysed how well individuals within each sample could be classified back to their original sample using ONCOR (Anderson *et al.*, 2008), with the leave-one-out option. The test was made by removing fish from sample populations (one at a time) and then re-estimating their origin.

Finally, we used MLRelate (Kalinowski *et al.*, 2006) and all 5560 SNPs to test whether individuals within samples were related. ML-Relate calculates maximum-likelihood estimates of genealogical relationship and relatedness between individuals.

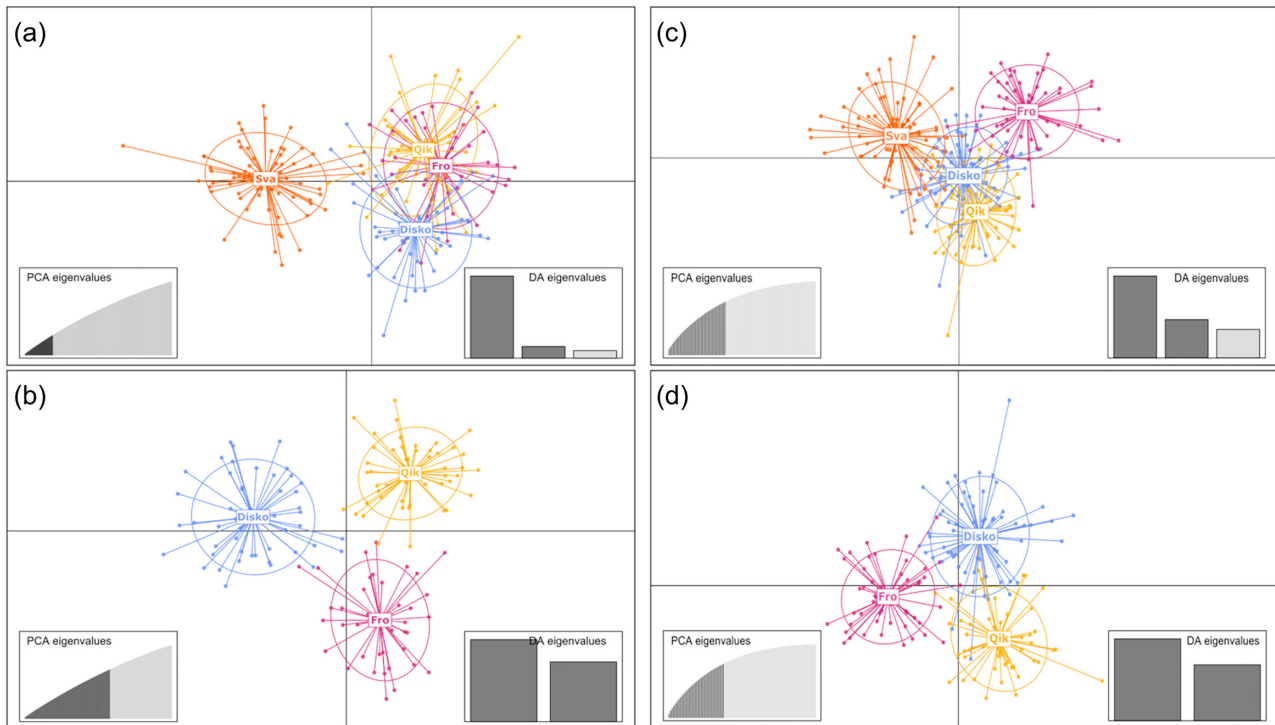


Figure 2. Plots of DAPC for (a) all juvenile samples using 5 560 SNPs, (b) for the west Atlantic juvenile samples only, (c) for all samples using 90 SNPs, and (d) for the west Atlantic juvenile samples only. Juvenile samples are shown by inertia ellipses and different shapes and colours. Each dot represents an individual. Sva: Svalbard, Disko: Disko Bay, Qik: Qikiqtarjuaq, and Fro: Frobisher Bay.

Results

A total of 210 fish were successfully genotyped for the 5 560 SNP loci. Initial analysis of population structure on the full set of SNPs using DAPC and STRUCTURE shows differentiation among juvenile samples. Forty principal axes and three DFs were retained in the DAPC analysis, and results showed a separation of Svalbard from the other samples, with an overlap between Disko Bay, Frobisher Bay, and Qikiqtarjuaq (Figure 2a). After we removed the Svalbard samples and re-ran the analysis, 80 principal axes and two (DF) characterized differences were observed among western Atlantic samples (Fro, Qik, and Disko), showing a clearer separation among them all (Figure 2b). STRUCTURE analysis found $K = 2$ as the most likely number of clusters, clearly separating the Svalbard samples from the NWA (Supplementary Figure S1a). Further, STRUCTURE analysis on only the three NWA samples found $K = 2$ as the most likely number of clusters where all individuals were admixed (Supplementary Figure S1b).

LOSITAN identified 90 SNP loci with large F_{ST} values, of which six were possibly under selection, but none was found under balancing selection (Supplementary Figure S2). BayeScan also found six outlier loci, which were not all the same but were within the ten loci with the largest F_{ST} , identified by LOSITAN (Supplementary Figure S3). The panel of 90 SNPs were then used for population genetic analysis ($n = 247$), including and excluding the 6 SNP outliers found in LOSITAN.

Observed locus heterozygosity (H_O) varied from 0.04 to 0.56, where seven loci had H_O over 0.5 (Supplementary Table S1). H_O among samples ranged from 0.318 for Frobisher Bay to 0.340 for Svalbard (Table 2). Test for linkage disequilibrium (LD) over all loci within samples indicated significance in 21 of 16 020 tests (0.0013) after correction for multiple tests, well under expectations at an $\alpha = 0.05$. Similarly, tests

Table 2. Observed (H_O) and expected (H_E) heterozygosity over all loci for each sample and inbreeding coefficient (F_{IS}) for each sample.

Sample	H_O	H_E	F_{IS}
Svalbard	0.340	0.314	-0.0075
Disko Bay	0.321	0.316	-0.0286
Frobisher Bay	0.318	0.307	-0.0757
Qikiqtarjuaq	0.322	0.311	-0.0249

over all loci and samples indicated significance in 11 tests of 4005 (0.0021). Results were similar for the dataset without outlier loci. The LD test over all loci within samples found significance in 10 of 13944 tests (0.0007), after correction for multiple tests, and the LD test over all loci and samples found significance in 8 of 3486 (0.0023) significant tests. The P tests for HWE for each sample and locus indicated that Svalbard was significantly out of HWE at one locus, whereas the other samples were in HWE for all loci after correction for multiple tests (Table 2), which did not change in the datasets without the outlier loci.

A significant level of pairwise F_{ST} was found between all samples (Table 3). A clear geographic pattern was observed with the largest differences found between Svalbard and the three western locations. Considering only NWA samples, Frobisher Bay showed greater genetic differentiation relative to Disko Bay and Qikiqtarjuaq (0.025 and 0.027, respectively), compared to that observed between Disko Bay and Qikiqtarjuaq (0.018). The genetic differences were similar and highly significant, excluding the six outlier loci.

For the DAPC, 30 principal axes and three DFs were retained, which separated all samples, but with some overlap between Disko Bay and Qikiqtarjuaq (Figure 2c). Re-running the analyses once Svalbard samples were removed produced

Table 3. Pairwise Weir and Cockerham (1984) genetic distances (F_{ST}) for the 90 SNP datasets below diagonal and without the six outlier loci above diagonal.

Sample	Sva	Disko	Fro	Qik
Sva		0.034	0.057	0.039
Disko	0.041		0.026	0.018
Fro	0.063	0.025		0.028
Qik	0.046	0.018	0.027	

Sva: Svalbard, Disko: Disko Bay, Qik: Qikiqtarjuaq, and Fro: Frobisher Bay. All tests were highly significant: $P < 0.0000$ after correction for multiple tests.

34 principal axes and two DFs, which better characterized differences among remaining samples (Fro, Qik, and Disko) and showed clear separation among them (Figure 2d).

Results of Bayesian clustering analyses using STRUCTURE were similar to those from the DAPC. The optimal number of clusters (K) based on the Evanno test suggested three clusters (Table 3). However, results from MedMed K , MedMean K , MaxMed K , and MaxMean K , (Supplemental Figure S4) (Puechmaile, 2016) all indicated $K = 4$ as the most likely number of clusters (Figure 3a). STRUCTURE results were not different when outlier loci were removed (Figure 3b).

Construction of a UPMGA tree using Nei's genetic distances (D_A) showed strong agreement with the other analyses of genetic population structure in this species (Figure 4). Support for branching at each node was large after 100 bootstraps over loci, offering good support for the observed population genetic structure.

Analysis of how well individuals are classified back to their original sample using ONCOR showed that individuals have high probabilities of belonging to their original populations. Individuals were correctly classified into populations in 74.4–89.7% of cases (Table 4). Analysis of genealogical relatedness between individuals within samples conducted in MLRelate showed that there were possibly four pairs of half sibs (HS) and 1 pair of full sibs (FS) in Svalbard, one pair of HS in Disko Bay, no related individuals in Frobisher Bay, and two HS in Qikiqtarjuaq.

Discussion

Our study demonstrates significant genetic differences between samples of juveniles from the northeast and northwest Atlantic. Our findings of significant population structure

within the NWA are novel. Juveniles sampled in Disko Bay, Frobisher Bay, and Qikiqtarjuaq are all assumed to originate from the Davis Strait spawning ground, but here, they demonstrate differences that could not be explained by coincidence. These results may indicate some level of philopatry in Greenland halibut even on a scale smaller than previously thought or observed. Prior studies on population genetic structure of Greenland halibut using various genetic markers have found a genetic differentiation between northeast and northwest Atlantic populations (Knutsen *et al.*, 2007; Westgaard *et al.*, 2017); however, in many studies, no population structure has been found in the NWA, suggesting panmixia (Knutsen *et al.*, 2007; Roy *et al.*, 2014; Westgaard *et al.*, 2017; Ferchaud *et al.*, 2022; but see Pomilla *et al.*, 2008; Carrier *et al.*, 2020). Recent studies have found that the Estuary/Gulf of St. Lawrence represents a single stock that differs genetically from the Atlantic around Newfoundland, which supports population structure in the NWA (Carrier *et al.*, 2020; Ferchaud *et al.*, 2022).

Our approach was similar to Carrier *et al.* (2020), by collecting juvenile fish at expected nursery areas under the assumption that these would be fed by nearby spawning grounds, to assess whether genetic structure exists in Greenland halibut before they initiate migration (Vihtakari *et al.*, 2021). However, spawning areas in the NWA are not well defined. Juveniles have been found on the slopes of the banks west of Disko Bay and on the northern and western slopes of Store Hellefisk Bank. Greenland halibut tend to move deeper as they grow and mature (Jørgensen, 1997); thus juveniles collected on these banks are likely to have recently settled and could be in close proximity to the spawning grounds. This sampling strategy targeting assumed juvenile nursery habitats in the Davis Strait/Baffin Bay region uncovered previously unreported genetic structure within the NWA Greenland halibut distribution.

For population structure to be identified within the NWA, spawning fish must return to their natal spawning grounds with some expected level of gene flow restriction among them. In effect, this suggests that young Greenland halibut in nursery areas initiate migration at a critical size (35 cm) and continue moving to deeper waters and migrating until mature. Once mature, they then return to natal spawning grounds.

Our selection of 90 SNPs with the largest F_{ST} from 5 560 SNPs has sufficient resolution to find population genetic structure among groups of juvenile Greenland halibut. The 90 SNP markers included six outlier loci assumed to be under selec-

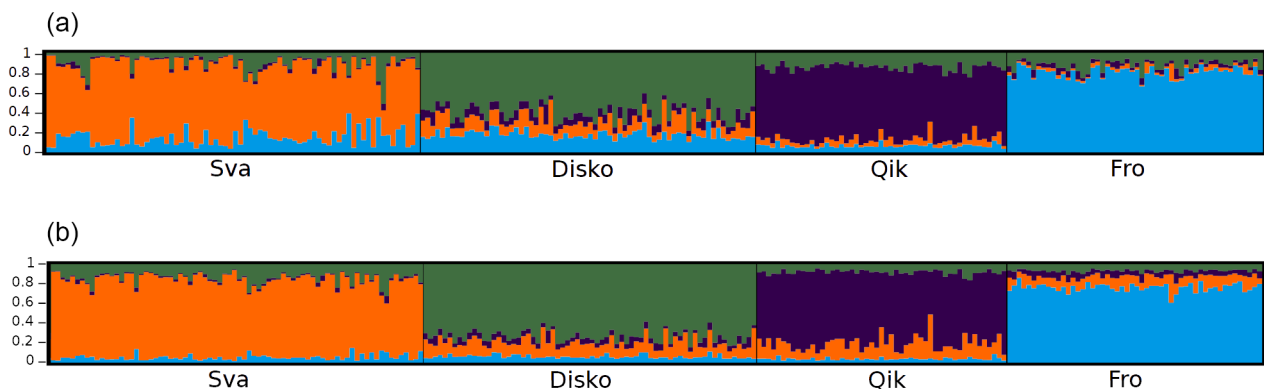


Figure 3. Bayesian cluster analysis carried out in STRUCTURE using (a) the dataset with 90 SNPs for $K = 4$, and (b) the dataset without the six outlier SNPs for $K = 4$. Sva: Svalbard, Disko: Disko Bay, Qik: Qikiqtarjuaq, and Fro: Frobisher Bay.

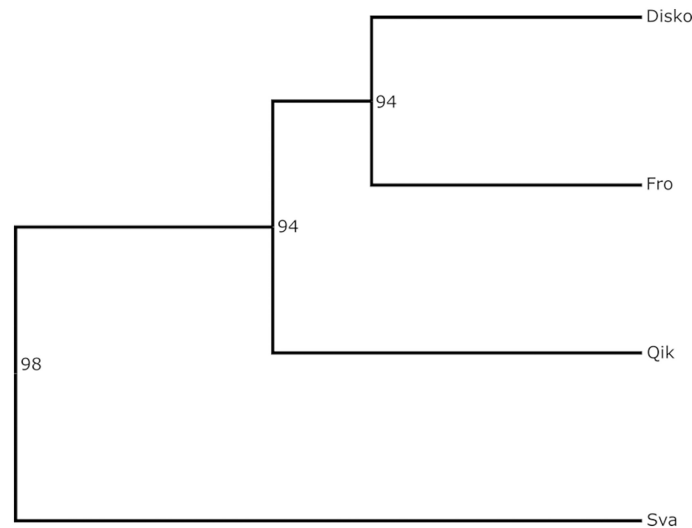


Figure 4. UPGMA tree constructed from Nei D_A genetic distances between four samples of Greenland halibut. Number at branches indicates bootstrap support after 100 bootstraps over loci. Sva: Svalbard, Disko: Disko Bay, Qik: Qikiqtarjuaq, and Fro: Frobisher Bay.

Table 4. Results of the leave one out test conducted in ONCORE to determine how well individual fish can be assigned to their population of origin.

Sample	% Correct	Largest misidentification
Sva	89.7	Disko 8.6%
Disko	76.3	Sva 10.5%
Fro	78.1	Disko 12.5%
Qik	74.4	Disko 16.3%

% Correct: % of correct identification to population. Largest misidentification: % of individuals that had the highest misclassification to other populations. Sva: Svalbard, Disko: Disko Bay, Qik: Qikiqtarjuaq, and Fro: Frobisher Bay.

tion. The six outlier loci found using BayeScan were not the same as those found by LOSITAN but were all within the top ten loci with the largest F_{ST} . However, when these six SNPs identified by LOSITAN were removed, we observed similar results. For our analysis, we used all 90 SNPs because tests confirmed adherence of the loci to HWE in all but the Svalbard sample, where two loci did not. Further, LD was weak and did not include loci within the different samples. Our tests for genotypic differentiation using F_{ST} were significant between all samples. Although the F_{ST} values are relatively small compared to genetic differences found in philopatric species such as Atlantic salmon, they are nevertheless larger than F_{ST} calculated for other highly fecund and dispersing marine species (Gagnaire *et al.*, 2015). F_{ST} values in this study are an order of magnitude larger than those found in recent studies of genetic structure in Greenland halibut using a similar or greater number of SNP markers (Westgaard *et al.*, 2017; Carrier *et al.*, 2020; Ferchaud *et al.*, 2022). In these later studies, samples were collected over a broad spatial scale and showed overall significant F_{ST} values ranging from 0.0014 to 0.003, relative to an F_{ST} of 0.038 calculated here.

In this study, we found six outlier SNPs possibly under divergent selection. However, each sample conformed to HWE, and there was very weak LD among loci. This would be unexpected if many of the used loci were under divergent selection (Gagnaire *et al.*, 2015). Thus, the 90 SNPs used here are most likely neutral, except perhaps for the six identified as outliers by LOSITAN. Although not investigated, it would be

interesting to relate the six outlier loci to environmental variation between the northwest and northeast Atlantic or within the Davis Strait. Ferchaud *et al.* (2022) found adaptive loci and significant differences between samples of Greenland halibut from the Gulf of St. Lawrence and the remainder of the NWA. The Gulf of St. Lawrence seems to differ strongly in salinity and temperature from areas outside the Gulf, making for an important selection gradient. Further, even if some of the loci are under adaptive selection and the genotypic differences between samples are not caused by genetic drift within populations, our findings of significant genotypic differences between samples still stand.

We investigated how well individuals could be assigned back to their population of origin using ONCOR. The test confirmed that the samples came from different populations albeit admixed, as we observed that 74.4–89.7% of individuals were assigned to their original samples. That there is some degree of admixture is to be expected considering Greenland halibut are highly migratory (Vihtakari *et al.*, 2022). Results thus suggest mixed-stock analysis to assign fish to their respective stocks may be possible for NWA fisheries.

All our samples were collected in the same year (2019). Consequently, there would be a possibility that observations and results show ephemeral genetic differences based on one or few cohorts that could change or disappear entirely if sampled in different, or over many, years. Carrier *et al.* (2020) showed a large turnover rate in terms of the genetic structure of individuals in expected spawning areas in the Gulf of St. Lawrence. Such temporal structure, however, is likely to apply only to differences observed in the NWA as other studies found genetic differences between NWA and northeast Atlantic samples (Westgaard *et al.*, 2017). Even so, temporal differences are unlikely because our samples were not from a single cohort but composed of multiple year-classes as fish of sizes <35 cm were used. We also investigated the possibility that individuals in each sample were closely related based on differences between family groups. This was not supported in the analysis of genealogical relatedness among individuals within samples conducted using MLRelate.

Our results indicate multiple spawning components in Greenland halibut in the NWA. Although the locations of

these putative spawning grounds remain unknown, we found large significant genotypic differences between samples of juveniles collected in nursery areas. Hence, the spawning grounds near the Davis Strait may be a composite of more genetically distinctive subpopulations. Greenland halibut spawn in the benthopelagic zone, where eggs and larvae drift along predominant currents. Just before hatching, egg specific density decreases, suggesting that larvae will be found higher in the water column (Domínguez-Petit *et al.*, 2013). Hence, larvae are transported by surface currents in the Davis Strait, which flow northwards with the west Greenland Current along the west Greenland coast, and southwards with the Labrador current along southern Baffin Island and Labrador before the larvae settle to the bottom. Because of egg and larva movement via oceanic currents to nursery habitats, juveniles are not expected to be found on spawning grounds that are yet to be defined. Future work should sample juvenile habitats over multiple years to confirm whether the genetic structure observed here is stable, as the trajectory of drifting eggs and larvae could change based on differences in the strength and direction of oceanic currents and spawning times.

Conclusions

In this study, we assumed that young fish sampled in juvenile nursery habitats could be used to identify spawning populations because spawning fish are seldom observed, particularly in the NWA. The present study found both an east–west population genetic structure across the North Atlantic, but also more complexity in structure in nursery areas adjacent to the Davis Strait, which has been assumed to be a single spawning ground. Results suggest the spawning of different genetic components is taking place near the Davis Strait. The present results call for further studies on the temporal stability of genetic structure seen here, modelling of egg and larva transport and movement of juveniles from spawning sites, and whether these corroborate observed genetic structure.

Acknowledgements

For facilitating sampling of Greenland halibut: Institute of Marine Research (IMR) Surveys collected the Norwegian juveniles, Greenland fishers collected the juvenile samples from Disko Bay, while in Canada, the juveniles were collected during Fisheries and Oceans Canada inshore research surveys conducted in collaboration with the Government of Nunavut. Thanks to the sequencing facility at AgResearch Limited, and team leader Rayna Anderson, Invermay Agricultural Centre, New Zealand, lab, for help and advice on the processing of the GBS data.

Funding

The project was supported by the NORSUSTAIN project funded by the joint Danish, Greenlandic, and Faroese Presidency of the Nordic Council of Ministers in 2020 and IMR project deep-sea species (grant no. 15179).

Conflict of interests

There is no conflict of interests.

Supplementary material

Supplementary material is available at *ICESJMS* online version of the manuscript.

Authors' contributions

T.J., S.S., D.G., J.B., M.T. D.E-B. designed the study. D.G., S.S., D.E-B., and A.H. conducted data analysis. D.G., T.J. S.S., D.E-B., D.R. wrote the manuscript and all authors contributed to editing and revising the manuscript.

Data availability

Raw reads will be uploaded to the NCBI short read archive when published. Files with genotypic information for all individuals and all scripts used in this study will be available on OSF at https://osf.io/z34n8/?view_only=9dffcb76c7b041d89ae44127d7f6b163.

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