A putative structural variant and environmental variation associated with genomic divergence across the Northwest Atlantic in Atlantic Halibut

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Abstract

Characterizing the nature of genetic differentiation among individuals and populations and its distribution across the genome is increasingly important to inform both conservation and management of exploited species. Atlantic Halibut (*Hippoglossus hippoglossus*) is an ecologically and commercially important fish species, yet knowledge of population structure and genomic diversity in this species remains lacking. Here, we use restriction-site associated DNA sequencing and a chromosome-level genome assembly to identify over 86,000 single nucleotide polymorphisms mapped to 24 chromosome-sized scaffolds, genotyped in 734 individuals across the Northwest Atlantic. We describe subtle but significant genome-wide regional structuring between the Gulf of St. Lawrence and adjacent Atlantic continental shelf. However, the majority of genetic divergence is associated with a large putative chromosomal rearrangement (5.74 megabases) displaying high differentiation and linkage disequilibrium, but no evidence of geographic variation. Demographic reconstructions suggest periods of expansion coinciding with glacial retreat, and more recent declines in N_e. This work highlights the utility of genomic data to identify multiple sources of genetic structure and genomic diversity in commercially exploited marine species.

Introduction

Identifying the genomic basis of population structure and local adaptation is a fundamental goal of both evolutionary biology (Savolainen *et al.,* 2013) and conservation genetics (Funk *et al.,* 2012), and is key to delineating and conserving intraspecific diversity. Detecting this variation in marine species has been challenging due to low levels of differentiation, large population sizes, and high connectivity frequently uncovered in marine organisms, which have often precluded the resolution of significant population divergence using neutral genetic markers (Hauser and Carvalho 2008; Gagnaire *et al.* 2015). However, with expanded availability of massively parallel sequencing, genomic analyses of population structure in marine species have increasingly revealed diverse and complex signatures of population differentiation (Lamichhaney *et al.,* 2012, 2017; Van Wyngaarden *et al.,* 2017). These patterns range from genome-wide polygenic variation associated with subtle, coordinated shifts in allele frequency at multiple loci (Le Corre and Kremer 2012; Bay and Palumbi 2014; Babin *et al.* 2017) to localized genomic regions housing structural variants or genes of large effect within otherwise undifferentiated genomes (Prince *et al.,* 2017; Kess *et al.,* 2019; Longo *et al.,* 2020). Across studies, genomic differentiation has been found associated with environmental variation (Bradbury *et al.,* 2010; Lamichhaney *et al.,* 2012; Stanley *et al.,* 2018), spawning time (Lamichhaney *et al.,* 2017) and behavioural traits (Prince *et al.,* 2017; Kess *et al.,* 2019). These observations support the hypothesis that genetic structuring in marine species often underlies adaptive differences that delineate significant ecological diversity, revealing adaptation at multiple geographic scales. Uncovering the genetic architecture underlying environmentally-associated and adaptive genetic variation can inform how distinct populations should be managed and conserved (Funk *et al.,* 2012), and increasingly can enable examinations of past and future responses to environmental change (Bay *et al.,* 2017; Lehnert *et al.,* 2019; Layton *et al.,* 2021).

Recent methodological advances have enabled estimation of demographic history in non-model species, allowing identification of historical changes that have impacted populations (Barbato *et al.,* 2015; Liu and Fu 2015; Hollenbeck *et al.,* 2016). Observations from these studies have uncovered correspondence between climate shifts or potential human impacts, and perturbations to species demographic histories (Cristofari *et al.,* 2018; Vijay *et al.,* 2018; Lehnert *et al.,* 2019). Past changes in demography can have far-reaching impacts on contemporary diversity and shed light on factors shaping adaptive variation within species (Mitchell-Olds *et al.,* 2007; Ellegren and Galtier 2016). Pairing estimates of genetic diversity with demographic reconstruction methods can reveal how historical

forces have shaped the diversity of contemporary populations and can further aid in understanding the adaptive potential of studied species (Ruzzante *et al*., 2008; Wang *et al*., 2017).

Atlantic Halibut (*Hippoglossus hippoglossus),* a migratory, cold-water, and economically valuable groundfish species (DFO 2018), is currently managed in Canada as two distinct stocks: one stock on the Atlantic continental shelf, spanning the Scotian Shelf and the southern Grand Banks in NAFO divisions 3NOPs4VWX5Zc, and a second in the Gulf of St. Lawrence spanning NAFO divisions 4RST (DFO 2015a, 2015b). There is significant heterogeneity in stock status across the northwest Atlantic, with recent and historical examples of population decline in US stocks contrasting dramatic increases in Canadian waters (Grasso 2008; Shackell et al. 2016; Trzcinski and Bowen 2016). Despite its commercial and ecological importance, there is little understanding of how existing management units correspond to genomic differentiation or adaptive diversity within this species. Atlantic Halibut exhibit regional differences in life history, suggesting the potential for cryptic fine-scale genetic differentiation among populations across the Northwest Atlantic (Armsworthy and Campana 2010; Shackell *et al.,* 2019) but any genetic basis for these differences remains unknown, with essentially no population structure detected using microsatellite loci (e.g., Reid *et al.,* 2005). The genomic consequences of demographic change, and the level of contemporary diversity in Atlantic halibut populations also remains unknown, limiting understanding of how this species has responded to past disturbance or could respond in the future to ongoing climate change. Genomic methods have the capacity to reveal cryptic structure in exploited marine species (Selkoe *et al.,* 2016; Kelley *et al.,* 2016), and application of genomic tools to quantify population structure is integral to informing the conservation and management of Atlantic Halibut in the Northwest Atlantic.

In this study, we use genome-wide single nucleotide polymorphisms (SNP) obtained by restriction-site associated DNA (RAD) sequencing (Baird *et al.,* 2008) to investigate population genomic structure, diversity, and demographic history in Atlantic Halibut sampled across the Northwest Atlantic. Specifically, we (1) examine population structure across the Northwest Atlantic, and quantify divergence associated with a large putative chromosomal rearrangement, newly identified in this study, (2) explore the presence of environmental associations with population structure across the Northwest Atlantic, potentially indicative of adaptation, and (3) reconstruct the demographic history of Atlantic Halibut over the past 150,000 years and explore temporal associations with climate. Our findings here support a role for environmental variability in shaping genetic diversity in marine species, and provides empirical support for subtle genome-wide divergence in the marine environment.

Methods

Sampling & Sequencing

We obtained tissue samples from Atlantic Halibut caught between 2017 and 2018 on Department of Fisheries and Oceans (DFO) Canada trawl surveys, DFO-Industry longline surveys, Maine Department of Marine Resources trawl surveys, Nature Conservancy-Cape Cod industry samples, and Spanish Institute of Oceanography surveys in NAFO regions outside the Canadian Exclusive Economic Zone in NAFO Divs. 3N, 3O, and on the Flemish Cap (See Table 1 for location and sampling details) distributed across the Northwest Atlantic (Figure 1). Tissue samples were taken from fin clips, hearts, or spleens of sampled fish, and preserved in 95% ethanol. Sex was inferred from the visual identification of reproductive organs during dissection of sampled fish.

DNA was extracted from tissue samples using Qiagen QIAamp 96 DNA QIAcube HT Kit and QIAamp DNA Mini Kit extraction kits and assessed for DNA quality using Quant-iT PicoGreen. Following DNA extraction, RADseq libraries were constructed from 768 individual tissue samples passing DNA quality thresholds. Libraries were developed following an adapted and optimized version of the Ali *et al.,* 2016 protocol without the use of bait sequences to recover specific genomic regions, following the method described as "New RAD" in the initial publication and referred to as "bestRAD" in subsequent studies (e.g. Rochette et al. 2019). For each library, we randomized 48 individuals across regions, digested DNA using the *SbfI* restriction enzyme, and then ligated 48 unique RAD-Cap barcodes to each individual within a library. We then pooled, purified, and sheared DNA from these uniquely barcoded individuals, followed by isolating sheared RAD-tagged DNA for each pool. Library preparation then followed NEBNext Ultra DNA Library Prep for Illumina with no modifications. Each library received a unique index (1-16) which corresponded with the library number (e.g., Library 1 had index 1). Pooled libraries of 48 uniquely-barcoded individuals were sequenced on 16 separate lanes of an Illumina HiSeq 4000, using paired end sequencing of 100bp reads, at the McGill University and Génome Québec Innovation Centre.

Read Processing and SNP calling

Read quality for each library was checked using FastQC (Andrews et al. 2010) and we then used cutadapt 2.1 (Martin 2011) to trim all reads to a minimum length of 90 bp and remove adapter read-through sequence. Reads were then demultiplexed using the *process_radtags* function in Stacks 2.5 (Rochette *et al.,* 2019), using the *bestrad* flag, a barcode distance of 2, and removing reads with uncalled bases or falling below the default quality score. We then aligned all paired, demultiplexed

reads to the Atlantic Halibut Primary Curated reference genome (Einfeldt *et al., in prep,* available at https://vgp.github.io/genomeark/Hippoglossus_hippoglossus/), using the Burrows-Wheeler algorithm in *bwa mem* 0.7.17 (Li 2013), and sorted aligned reads using SAMtools 1.9 (Li *et al.,* 2009). We used *gstacks* in Stacks 2.5 to remove PCR duplicates with the *–rm-pcr-duplicates* flag and call RAD loci and SNPs, and then used *populations* to export a variant call format (vcf) file of SNPs using the *–vcf* flag, filtered for minor allele frequencies of 0.01 (*--min-maf* 0.01) with a minimum genotyping rate of 0.8 (*-r* 80). We then used *vcftools (*Danecek *et al.,* 2011) to remove SNPs not aligned to the 24 large chromosomal scaffolds, or with a minimum depth below 15 reads. We tested SNPs for deviation from Hardy Weinberg equilibrium (HWE) in plink 1.9 (Chang *et al.,* 2015) and calculated false discovery rate *q* values (Storey and Tibshirani 2003) for significant HWE deviation using the R package *qvalue* (Storey 2015). We then removed SNPs in autosomal regions with *q* values less than 0.05 in both the Atlantic continental shelf region as well as in the Gulf of St. Lawrence. Initial population structure analysis with *pcadapt* (Luu *et al.,* 2017) revealed a single large cluster containing the majority of individuals (n = 751), and 12 individuals separated on the first four PC axes (Supplementary Figure 1), but a low overall proportion of genetic variation explained (< 1%). We conducted separate Identity By Descent (IBD) analyses in plink using the *genome* function on these 12 individuals and the set of 751 individuals which did not reveal aberrant PCA clustering, and found high pi-hat values (>0.975) among 8 individuals. Comparisons among individuals in this set also showed elevated pi-hat values overall (mean pi-hat = 0.3) relative to the larger dataset of 751 individuals (mean pi-hat = 0.004). Together, these differences in IBD and PCA clustering indicate potential genotyping of duplicate samples, closely related individuals, or those from another lineage (Stevens *et al.,* 2011). These individuals were removed from subsequent analyses (n = 12). We then removed individuals that had incomplete sampling information from DFO surveys (n = 17). These filtering steps resulted in a final dataset of 86,043 SNPs distributed across the genome in 734 individuals.

To explore the impact of removing linked loci on inference of population structure, we also produced three additional panels of SNPs: 1. by removing chromosomes S15 and S18 due to identification of a putative chromosomal inversion on chromosome S15 (see below) and a sex-associated region on chromosome S18, found by PCA clustering of the initial 86,043 SNPs, which impacted detection of geographic population structure; 2. by filtering for linkage disequilibrium (LD) to generate an LD-pruned panel through removal of SNPs with $r^2 > 0.5$ in 50 SNP sliding windows, analyzed iteratively in steps of five SNPs s using plink, and removing chromosomes S15 and S18; 3. a panel of SNPs with a single SNP per RAD locus retained, and removing S15 and S18. A breakdown of

the number of SNPs, RAD loci, and proportion of SNPs mapping to each strand orientation at each stage of filtering are provided in Table 2.

Range-wide Population structure and Genetic differentiation

We first examined population structure across the Gulf of St. Lawrence and Atlantic continental shelf using principal component analysis (PCA) in the R package *pcadapt* . We conducted PCA using all SNPs 86,043 SNPs, followed by PCA using each of the subset SNP panels (i.e., filtered for LD and with single SNPs per RAD retained, with chromosomes S15 and S18 removed). We then identified SNPs significantly associated with population structure based on Mahalanobis distance of correlations with K = 3 PC axes, selected based on absence of genetic structure detected on subsequent axes, which instead separated individual samples. We then calculated *q* values for each SNP, and selected outliers as those SNPs with significant associations with K = 3 PC axes with $q < 0.05$. To test for within-region genetic variation, we conducted PCA using individuals sampled from the Atlantic continental shelf (N=521 individuals) and within the Gulf of St. Lawrence (N=213 individuals) separately, and a set of 62,213 SNPs with chromosomes S15 and S18 removed to prioritize detecting spatial structure rather than the putative rearrangement or sex locus.

We calculated ancestry proportions for each individual using the sparse non-negative matrix factorization (*snmf*) function in the *R* package *LEA* (Frichot *et al.,*2014; Frichot and Francois 2015) using the panel of 62,213 SNPs with S15 and S18 removed, to prevent assignment of ancestry to individuals based on putative inversion or sex chromosome variation. We investigated K = 1-5 sources of ancestry, tested for the number of significant sources of genetic ancestry using cross-entropy criteria, and repeated this analysis with the SNP panels filtered for LD, and with a single SNPs per RAD locus retained.

We calculated Weir and Cockerham's (1984) F_{ST} in plink between samples obtained from the Gulf of St. Lawrence and the Atlantic continental shelf and assessed significance of F_{ST} values in the R package *StAMPP* using 500 bootstraps (Pembleton *et al.,* 2013) using the full dataset of 86,043 SNPs. This grouping was selected based on subtle genetic differentiation observed between these regions using *pcadapt,* as well as the current management regime for Atlantic halibut in Canadian waters. To identify SNPs associated with subtle genetic structuring between regions, we selected the top 1% of SNPs based on F_{ST} as outliers. We then explored the utility of these SNPs in delineating population structure as a possible method to identify structure despite low overall genomic differentiation (Gagnaire *et al.* 2015) by repeating *pcadapt* and *snmf* analyses using only F_{ST} outlier SNPs. Last, to test for significant differentiation within the Atlantic continental shelf, we compared F_{ST} between

individuals from the most distant regions of this sampling area. We estimated F_{ST} between 60 individuals from the Gulf of Maine and 60 individuals from Newfoundland and Grand Banks, and assessed significance using *StAMPP*.

Environmental association

We calculated associations between SNP genotype and environmental variables to identify the role of oceanographic features in driving population structure and genomic differentiation. First, we extracted environmental values averaged over all months and years from 2000 to 2014. These variables included maximum, mean, and minimum salinity, dissolved oxygen content, and temperature at mean bottom depth for the sampling location of each individual, obtained from the Bio-Oracle 2.2 database (Assis *et al.,* 2018), using the R package *sdmpredictors* (Bosch 2018). To reduce the number of correlated variables tested for environmental association, we standardized each variable by its standard deviation and then conducted principal component analyses separately on temperature, salinity, and oxygen variables to produce three principal components (Supplementary Figure 2). We used the first axis of each principal component as aggregate measures of temperature, salinity, and dissolved oxygen. Pearson's correlation coefficients among each of these PCs were below 0.7 in all pairwise comparisons, and thus we retained all three of these PCs as predictors for environmental association. We then conducted redundancy analysis (RDA, Rao 1964) with the *vegan* R package (Oksanen *et al.,* 2011), using principal components of environmental variables as predictors and SNP genotypes as response variables. We also conducted a partial RDA that was first constrained by latitude and longitude values to assess the role of environment independent of geography in generating genomic differentiation. Last, the significance of geography alone was assessed through an RDA on only geographic coordinates. The significance of each model was assessed using 999 permutations with the *anova.cca* function.

To identify SNPs significantly associated with environmental variables, we used the *Rdadapt* R function (Capblancq *et al.,* 2018, Supporting Information Script S1) to calculate Mahalanobis distances of z-transformed canonical axis loadings on all three canonical axes, selected based on visualization of Scree plots. This method allows for a statistical test of significance of association of SNPs with environmental variables, by computing per-SNP *p* values estimated from Mahalanobis distances for each SNP, which can then be adjusted as *q* values for False Discovery Rate. Here, we used a conservative approach to identify outlier SNPs as those with a q value < 0.05 and canonical axis scores > the 99.9th percentile of a canonical axis. We then compared the number of environmental outlier SNPs detected from RDA and RDA with constraint by geographic variation.

Characterization of a potential structural variant

We identified a highly divergent genomic region on chromosome 15 in *pcadapt* comparisons consistent with a potential structural variant. To quantify divergence between different genotypes heterozygous or homozygous for either allele of this genomic region, we separated individuals into genotype groups based on PC1 score from PCA restricted to chromosome 15. We then estimated F_{ST} between homozygous genotype groups. To quantify linkage disequilibrium (LD), we estimated pairwise r^2 between all SNPs on chromosome 15 in plink and estimated mean r^2 for each SNP with all adjacent SNPs in 50 SNP windows. We then calculated Pearson correlation coefficients for LD (r^2) and F_{ST} for SNPs within and outside of the putative structural variant. Next, we estimated heterozygosity per SNP for each genotype group, as well as LD in 50 SNP windows for each genotype group, and used Mann Whitney U Tests to compare significance of LD and heterozygosity estimates within and outside the putative rearrangement. We then carried out a test of Hardy Weinberg Equilibrium in plink using assigned putative inversion genotypes as a single locus.

We conducted an additional test for the presence of a structural variant on chromosome 15 using the R package *inversion* (Cáceres et al., 2012), which uses patterns of local LD to infer and phase inversion breakpoints, assuming the population consists of both inverted and non-inverted haplotypes, with inversion size inferred from patterns of contiguous LD.

To identify potential molecular functions of this region we extracted annotation information from the coordinates of the divergent region from chromosome NC_047158.1, which corresponds to S15 in the Primary Curated reference used for SNP calling, in the annotated GCA_009819705.1 Atlantic Halibut assembly (available at https://www.ncbi.nlm.nih.gov/assembly/GCA_009819705.1), using the .gff format annotation and using bedtools (Quinlan and Hall 2010). We then extracted all annotated genes with gene symbols from aligned regions, and conducted a gene ontology overrepresentation test of all biological and molecular terms using the *Danio rerio and Homo sapiens* genomes for separate comparisons in PANTHER (Mi *et al.,* 2019), using a Bonferroni adjusted alpha of 0.05 for significant terms.

Genetic diversity and demographic history

We estimated genetic diversity and demographic history separately in Gulf of St. Lawrence and Atlantic samples, based on separate management regimes and genomic differentiation between these sites. We estimated per-locus observed (H_o) and expected (H_e) heterozygosity using plink. We then randomly chose 60 individuals from each region and downsampled to 1000 un-linked SNPs with minor

allele frequency > 0.05 to calculate recent effective population size (N_e) for each region using NeESTIMATOR v 2.0 (Do *et al.,* 2014).

ANGSD 0.931 was used to export folded site-frequency spectra (SFS) for calculation of nucleotide diversity (π), Tajima's D, and demographic reconstruction in each region. We used the Picard 2.26 *MarkDuplicates* function and *IndelRealigner* function in Genome Analysis Toolkit v3.7.0 (DePristo *et al.,* 2011) to generate a set of sorted, indel-realigned, de-duplicated bam alignments for 200 individuals from the Atlantic continental shelf and 200 individuals from the Gulf of St. Lawrence. Site frequency spectra for all chromosomes, excluding S15 and S18, were exported for samples from the Gulf of St. Lawrence and from the Atlantic continental shelf separately using ANGSD 0.931 (Korneliussen *et al.,* 2014), and the reference genome as the ancestral genome, with the following flags: *-dosaf 1 -minMapQ* 30 *-minQ* 20 *-minInd* 200 *-doMajorMinor* 5 *-doMaf* 2 *-uniqueOnly* 1 *-remove_bads* 1 *-only_proper_pairs* 1. We generated SFS from 13,001,232 and 12,148,475 genotyped variant and invariant sites in the Atlantic continental shelf and Gulf of St. Lawrence samples, respectively. We then output folded site frequency spectra using the *realSFS* function with -fold 1. We used r*ealSFS saf2theta* with *fold* -1 to estimate per-site theta values and *thetaStat dostat* on folded SFS and estimated theta values to estimate Tajima's D separately for each region. To estimate π, we divided pairwise theta (tP) by the number of sites compared (nsites) for each chromosome, and then averaged this value over all chromosomes.

To estimate demographic history at different time scales, we used two demographic reconstruction methods. First, we used a coalescent method based on the site frequency spectrum (*stairwaytplot2*, Liu and Fu 2015; Liu and Fu 2020) to infer demographic history over long time periods. We then ran *stairwayplot2* using default recommended parameters for folded site frequency spectra, with mutation rates of 1.0×10^{-8}, which has frequently been used as a default rate in studies of fish species (Delrieu-Trottin *et al.,* 2020), and 2.0×10^{-9}, (Feng *et al.,* 2017), the measured mutation rate in Atlantic Herring, and among the lowest recorded in a fish species, and time to maturity of 9.5 years, based on averaged Atlantic Halibut time to maturity (Nielsen 1986). To estimate recent changes in demographic history, we also used the linkage-based method implemented in SNeP (Barbato *et al.,* 2015). This method infers recombination rates between markers based on physical position, and linkage between markers at varying recombination distances is then used to estimate changes in effective population size across time, with lower recombination rates between loci estimating older demographic events. Because measures of N_e from SNeP are strongly sample size dependent, we use this method to infer relative N_e across time only, by dividing each N_e estimate by maximum inferred N_e.

Results

Population structure and genetic differentiation

The strongest signals of genetic differentiation detected among individuals were associated with highly divergent regions on chromosome S15 and chromosome S18 (Figure 2A, Supplementary Figure 3A). These genomic regions correspond to a potential large structural variant (chromosome S15) and to a putative sex determining region on chromosome S18, based on clear separation of individuals on PC2 by phenotypically-assigned sex (Supplementary Figure 3, Supplementary Figure 4B, Einfeldt *et al.*, *in revision*). In PCA comparisons, the axis strongly associated with the divergent region on chromosome S15 explained 0.23% of genomic variation and was associated with clustering of individuals in three distinct groups, (Figure 2B, Supplementary Figure 4A), likely representing genotype combinations of two separate haplotypes. PC axis 2, primarily associated with sex differentiation, explained 0.22% of genomic variation (Supplementary Figure 3). We identified 986 outliers using *pcadapt,* 445 of which were found on chromosome S18, and 461 on chromosome S15, whereas 80 were on other chromosomes.

We also identified subtle genetic differentiation between the Gulf of St. Lawrence and Atlantic continental shelf (i.e., Scotia Shelf / Grand Banks regions). This differentiation was reflected in very low (F_{ST} = 0.0005, min SNP F_{ST} = 0, max SNP F_{ST} = 0.068) but significant ($p < 0.001$) divergence between regions, and clear separation of Gulf of St. Lawrence on PC axis 3 in the PCA analysis of the full dataset. The 861 SNPs in the top 1% of the F_{ST} distribution (mean F_{ST} = 0.018, max F_{ST} = 0.068) were found across all 24 chromosomes (Figure 3A). Using *snmf* on the dataset with chromosomes S15 and S18 removed, cross entropy criterion suggested only K =1 ancestral groups, indicating very low overall genetic divergence between regions. PC and *snmf* analyses with F_{ST} outliers uncovered divergence between the Gulf of St. Lawrence individuals and those collected on the Atlantic continental shelf, as well as greatest cross entropy criterion support at K =2 (Figure 3B, 3C).

PCA and *snmf* on datasets with chromosomes S15 and S18 removed, and filtered for LD or with single SNPs per RAD locus retained revealed similar patterns of separation in PCA (Supplementary Figure 5), cross-entropy criteria at K=1, and similar values of significant F_{ST} for both datasets (0.0005, $p < 0.001$). PCA within separate regions (Atlantic continental shelf, Gulf of St. Lawrence) did not reveal hierarchical spatial structure (Supplementary Figure 6 A,B), and F_{ST} comparisons between the most distant regions of the Atlantic continental shelf – Newfoundland and Grand Banks compared to the Gulf of Maine, did not reveal significant differentiation ($F_{ST} < 0.0001$, $p = 0.1$).

Environmental association

Redundancy analysis also identified subtle genomic differentiation between the Gulf of St. Lawrence and the Atlantic continental shelf associated with environmental variation between these regions (Figure 2C). Environmental variables explained a small but significant proportion of overall genomic variation ($p < 0.001$, adjusted $R^2 = 0.023\%$), with each environmental PC identified as significant in the model ($p < 0.001$). The first environmental axis showed strongest association with the PC of dissolved oxygen variables, inferred from similar direction on RDA1 axis of the vector of oxygen PCs (Figure 2C). The observed pattern of environmental association was genome-wide with 261 loci exhibiting significant environmental associations, despite small combined variation explained (Figure 2D). We found that 49 (19%) of these loci were also F_{ST} outliers divergent between the Gulf of St. Lawrence and the Atlantic continental shelf (Figure 3A), whereas only 9 (3.5%) overlapped with autosomal *pcadapt* outliers outside of the putative structural variant on chromosome S15. In contrast, repeating this analysis with constraint by Latitude and Longitude revealed no significant associations between environmental variation and genome-wide divergence. However, we identified 260 significant outlier loci from the geography-constrained RDA, 44 of which were found in common between both sets of environmental outlier loci from both partial and full RDAs (9.2%), but only two loci of the 44 shared between RDA approaches matched F_{ST} outliers. RDA using only Latitude and Longitude revealed a significant association of Latitude with genomic variation ($p < 0.001$), but also explained a small proportion of overall genomic variation (adjusted $R^2 = 0.032\%$).

Characterization of a putative structural variant

We identified individual variation on chromosome S15 that exhibited high divergence that did not correspond to geographic variation (Figure 4A, 4B), suggesting fine-scale genome architectural variation in Atlantic Halibut. Based on separation of individual scores on PC1 from PCA restricted to SNPs on chromosome S15 (Supplementary Figure 7), we assigned 349 individuals as homozygous for haplotype 1, 57 individuals homozygous for haplotype 2, and 328 heterozygous individuals. We found that these genotypes did not show significant deviation from HWE ($p = 0.11$). We found a sharp rise in linkage disequilibrium ($r^2 = 0.04$, Figure 4C, 4D) and divergence between individuals homozygous for each haplotype ($F_{ST} = 0.18$) in a large genomic region from approximately 5.75 to 12 Mbp (Figure 4B). These coordinates are rough estimates, as with a reference genome size of ~0.597 Gbp, we have only mapped 33,076 RAD loci, corresponding to coverage of one RAD locus per ~18,000 bp. LD and F_{ST} values were significantly ($p < 10^{-10}$, both tests) higher than the remainder of the chromosome ($F_{ST} = 0$, LD $r^2 = 0.024$). F_{ST} and linkage between SNPs also showed correlated patterns in the divergent region

(Pearson's $r^2 = 0.18$, $p < 10^{-6}$), whereas no relationship between these statistics was found across the remainder of the chromosome (Fig 4D). We found elevated LD among all genotype groups at this region compared to the remainder of the chromosome (Supplementary Figure 8B, $p < 0.001$ in all comparisons).Comparison of heterozygosity among individuals with different genotypes indicated a rise in heterozygosity among inferred heterozygotes in this region (Supplementary Figure 8B $p < 0.001$), whereas homozygous individuals exhibited significantly reduced heterozygosity within this region relative to the remainder of the chromosome ($p < 0.001$ in both comparisons). Using *inversion,* we found additional support for structural variation underlying this divergent region. This method indicated breakpoints for a putative inversion between 5.7 Mbp and 10.9 Mbp coinciding with the location of the divergent genomic region. Extracting gene information from the annotated genome revealed overlap with 232 annotated genes with gene symbols, but no significant overrepresentation of biological processes, (Supplementary File 1).

Demographic history and genetic diversity

Estimates of genetic diversity were similar in both the Gulf of St. Lawrence and on the Atlantic continental shelf ($H_e = 0.22$, $H_o = 0.21$, $\pi = 0.0021$) and the Gulf of St. Lawrence individuals ($H_e = 0.22$, $H_o = 0.21$, $\pi = 0.024$). We did not find significant differences between regions in either estimate of diversity. Estimates of LD-based N_e were also infinite in both regions. We identified small, negative Tajima's D estimates in both the Gulf of St. Lawrence (-0.07) and Atlantic continental shelf (-0.23).

Reconstructions of demographic history using using *stairwayplot2* suggest that expansion of Atlantic Halibut population sizes coincided with warming during the end of Last Glacial Period (~11,000 years ago). Using both upper (1.0 x 10-8) and lower bound mutation rates (2.0 x 10-9), we found a period of increasing N_e was observed between the Mid-Holocene Warm Period (~5000-7000 years ago) and the end of the Last Glacial Maximum (~19,000 years ago, Figure 5A,B). These reconstructions exhibit large confidence intervals and plateaus of inferred N_e over the recent past: ~2000 years for the upper bound mutation rate and ~5,000 years for the lower bound mutation rate. Estimates of demographic change from *SNeP* identified ongoing decline during the past ~7000 years (Figure 5C).

Discussion

In this study, we conduct the first large-scale investigation of genomic diversity in Atlantic Halibut using high-density genomic markers and uncover previously unrecognized genetic structure in

this species. We identify subtle genomic differentiation associated with divergence between the Gulf of St. Lawrence and the Atlantic continental shelf, overlapping with loci significantly associated with environmental gradients. In contrast to subtle oceanographic structuring, we find that the majority of genetic variation is attributable to a large divergent region consistent with a putative chromosomal inversion, variable among individuals across the Northwest Atlantic. Estimation of demographic histories revealed contrasting patterns of high N_e coinciding with the end of the Last Glacial Period, and declines over more recent time-frames. Together, these results indicate that Atlantic Halibut exhibits both genome-wide, environment-associated population structure and fine-scale individual variation associated with a divergent chromosomal region indicating multiple sources of genomic differentiation in this species.

Our analyses of population structure identified a subtle but significant pattern of population divergence between the Atlantic continental shelf and the Gulf of St. Lawrence. The small proportion of genetic variation explained, and low but significant F_{ST} observed here, are both consistent with weak structuring identified in other high gene flow marine species (Pespeni and Palumbi 2013; Babin *et al.,* 2017; Jiménez-Mena *et al.,* 2020). These findings contrast with a lack of divergence observed in a microsatellite study of the Northwest Atlantic (Reid *et al.,* 2005), highlighting the capacity of large panels of genomic markers to detect subtle population structure (Bradbury *et al.,* 2010; Gagnaire *et al.,* 2015; Benestan *et al.,* 2015). This observation is consistent with the hypothesis that in marine species with large effective population size, long timeframes may be required for appreciable genome-wide differentiation to accumulate by drift, reflected in significant F_{ST} values, even in instances of low or zero connectivity (Hauser and Carvalho 2008). Under these conditions, even low levels of dispersal will add to time required for differentiation, making identification of significant divergence in marine species challenging (Gagnaire *et al.,* 2015). We uncover significant differentiation between regions, despite high effective population size and limited time for contemporary populations structure to develop following deglaciation of the Northwest Atlantic (Reid *et al*., 2005). This observation suggests forces beyond drift have driven contemporary population structure in the Northwest Atlantic, and we find environmental variation significantly explains a small proportion of genomic variation in this species. Individual scores on RDA axes similarly revealed separation between Gulf of St. Lawrence and Atlantic continental shelf individuals, indicating oceanographic features may contribute to genetic divergence between these regions. This pattern was not significant after constraining RDA by geographic variation, indicating disentangling patterns of geographic separation and environmental variation may be difficult in this system. However, comparisons of differentiation between distant sites along the Atlantic continental shelf did not reveal significant differentiation, indicating some feature

specific to the Gulf of St. Lawrence has led to subtle but detectable divergence. These findings are concordant with the recent identification of relatively high site fidelity of Atlantic Halibut in tagging studies (den Heyer *et al*., 2012; Le Bris *et al.,* 2018) as well as low connectivity and independence of population dynamics in this species (Boudreau *et al.,* 2017).

Using a test of multi-locus environmental association, we uncovered a genome-wide basis of environmental association with many loci dispersed throughout the genome exhibiting association with temperature, oxygen, and salinity gradients. Environmental variation has been found to coincide with barriers to genetic exchange of locally adapted loci across marine species, leading to genomic divergence and population structure (Van Wynngarden *et al.,* 2017; Stanley *et al.,* 2018; Kess *et al.,* 2020). The identification of genome-wide environmental association in Atlantic Halibut matches recent findings of similar genomic signatures of adaptation to environmental gradients in other marine species (Bay and Palumbi 2014; Healy *et al.,* 2018; Rey *et al.,* 2020). Multiple environmental gradients have been found to dictate divergence and population structure, including temperature (Xuereb *et al.,* 2019, Benestan *et al.,* 2016), salinity (Healy *et al.,* 2018), and oxygen (Berg *et al.,* 2015). Here, we find that differences in available dissolved oxygen between the Gulf of St. Lawrence and the Atlantic continental shelf are associated with subtle genomic divergence, contrasting recent identification of winter bottom temperature driving multi-species clinal differentiation in the Northwest Atlantic (Stanley *et al*., 2018). Recent identification of similar genetic structure in Greenland Halibut (Carrier *et al.,* 2020), as well as low oxygen availability in the Gulf of. St Lawrence (Brennan *et al.,* 2016) suggest adaptation to hypoxia as a potential mechanism driving environmental associations observed in this study. We also uncovered overlap between loci significantly associated with divergence between the Gulf of St. Lawrence and the Atlantic continental shelf, indicating reduced gene flow between these regions may correspond to different environmental tolerances. Despite identifying many significantly associated loci, their association with environmental gradients was weak and differentiation was relatively low even at outlier loci, suggesting that environmental adaptation has manifested in small allele frequency shifts (Le Corre and Kremer 2012; Flaxman *et al.,* 2014), compared to other high dispersal marine species which exhibit large regions of genomic divergence (Lamichhaney *et al.* 2017; Kess *et al.* 2020). However, some caution is warranted in interpreting the role of environmental outliers identified here, as habitat and spawning-site fidelity have been identified across the Northwest Atlantic, and thus environmental outlier loci identified here may also represent loci differentiated between regions due to divergence covarying with the differing environments between these localities (Gatti *et al.,* 2020; Le Bris *et al.,* 2018).

We identified a region of large genomic divergence on chromosome S15 that explained the majority of genetic variation, and exhibited high linkage and F_{ST}, consistent with a chromosomal rearrangement. We find that this region meets several criteria consistent with indirect evidence of a chromosomal inversion: elevated divergence and LD in the total dataset, and reduced heterozygosity among homozygous individuals relative to heterozygous individuals (Merot *et al.,* 2020). This region was responsible for the primary axis of genetic variation among individuals; the correlated divergence among linked SNPs (Lotterhos 2019), suggests divergence of this locus as a single "supergene" (Thompson and Jiggins 2014). With widespread availability of high-quality genomes and genotype data, chromosomal rearrangements are increasingly being identified as important genomic architectural features underlying supergenes and associated with adaptive phenotypes in marine species, including migratory and spawning differences in Atlantic Cod (Kess *et al.,* 2019), Atlantic Herring (Lamichhaney *et al.,* 2017), and Rainbow Trout (Pearse *et al.,* 2019). Recent exploratory studies in migratory and high gene flow species have also identified evidence consistent with rearrangements, without obvious phenotypic or environmental links, as observed here (Cayuela *et al.,* 2020; Longo *et al,.* 2020). Rearrangements and inversions may be especially important in species with high dispersal capability, enabling trait divergence despite ongoing gene flow (Hooper and Price 2017), and consistent with fine-scale variation despite gene flow we find within-region variation in rearrangement genotypes. Future genome-wide association studies with behavioural and phenotypic variation in Atlantic Halibut may aid in clarifying the role of this putative rearrangement. Additionally, we present only indirect evidence for structural variation; this uncertainty may be resolved by future long-read sequencing which can directly characterize rearrangements.

We uncovered similar patterns of genetic diversity and large effective population size across both geographic regions, and predictions of similar demographic histories. Large contemporary effective population sizes, inferred from infinite LD-based N_e estimates, may partially be attributable to recent population expansions occurring in Canadian waters, but consistent large effective population sizes were inferred despite qualitative patterns of decline in our LD based reconstruction of demographic history, consistent with the potential for high fecundity in this species (Trzcinski and Bowen 2016). Demographic reconstructions in *stairwayplot2* revealed population expansion occurring between ~5000 and 19,000 years, depending on specified mutation rate, coinciding with either the end of the Mid-Holocene Warm Period (~5000-7000 years ago) or following the Last Glacial Maximum (LGM). Demographic changes coinciding with climatic variation may be due to changes in abundance of prey or competitors, or changes in availability of suitable habitat (Hoegh-Guldberg and Bruno 2010; Cristofari *et al.,* 2018; Vijay *et al.,* 2018). The expansion of N_e associated with transition to the

Holocene suggests that either changes in ocean temperature or deglaciation of suitable habitat in the Northwest Atlantic may have enabled population expansion.

Some caution is warranted in over-interpreting these results, as fluctuations in migration and population structure may also generate patterns of demographic change (Mazet *et al.,* 2016). Additionally, some uncertainty is expected in our results, as genome-level mutation and recombination rate estimates for Atlantic Halibut are not available. We compensate for these limitations by estimating recent demographic changes using linkage-disequilibrium, instead identifying evidence of potential declines during the last ~7000 years, coinciding with the beginning of the Mid-Holocene Warm Period. The discrepancy in observations from these two methods may reveal their sensitivity to demographic changes occurring at more recent or distant timeframes. *SNeP* is limited in utility to infer older demographic changes without very high-density SNP panels (Barbato *et al.,* 2015), and similar LD based methods can be downwardly biased towards detection of ongoing decline due to recent population declines (Hollenbeck *et al*., 2016), as those that have been documented in Atlantic Halibut reflecting recent extirpations due to over-fishing (Shackell *et al.* 2012; Trzcinski and Bowen 2016). Similarly, the large confidence intervals and plateaus in N_e over the last ~2,000-5,000 years from *stairwayplot2*, dependent on specified mutation rate, suggest low resolution of recent demographic history. Although the exact cause of historical changes in diversity cannot be inferred, their coincidence with changes in climate supports the hypothesis that the demographic history of Atlantic Halibut may have been impacted by past climatic fluctuations.

Our results here provide insight into the magnitude and geographic scale of genomic differentiation in marine species, and are directly applicable to the conservation and management of Atlantic Halibut in the Northwest Atlantic. Our observation of environment-associated genomic differentiation between the Atlantic continental shelf and the Gulf of St. Lawrence provides further support for independent management of these regions (e.g., DFO 2015a, 2015b). Our observations indicate subtle genetic differentiation between stocks matching known differences in behaviour and life history (den Heyer *et al*., 2012; Le Bris *et al.* 2018), suggesting some limits to genetic exchange between regions. The observed genome-wide environmental associations are consistent with adaptation to different environmental conditions (e.g. oxygen availability), and may further reduce gene flow between regions. Additionally, further examination of the link between ecologically-distinct phenotypes and the newly identify putative structural variant is suggested and may inform the conservation of key phenotypic diversity, as has recently been considered in other marine species (Waples 2018).

Conclusion

Our study provides the first evidence of genome-wide population structure in Atlantic Halibut, revealing genomic divergence between the Gulf of St. Lawrence and Atlantic continental shelf regions. We also identify a large putative structural variant displaying high differentiation and linkage disequilibrium, indicating the presence of a potential supergene. Estimates of demographic history indicate potential ongoing declines in this species, and a more distant population expansion coinciding with the end of the Last Glacial Period. Together, these results reveal multiple previously uncharacterized sources of genomic differentiation in Atlantic Halibut.

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Author Contributions

T.K., I.R.B., D.R., and P.B. designed the study. T.K and T.E. conducted data analysis. T.K. 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 on publication. Files with genotypic information for all individuals and a metadata file of individual location and environmental parameters will be uploaded to dryad on publication. All scripts used in this study are available at: https://github.com/TonyKess/Halibut_RADseq

Table 1. Sampled regions, sampling trips, number of samples per trip, time of sampling, and gear used to collect 768 Atlantic Halibut tissue samples used for RAD sequencing library preparation.

Region Sampled	Sampling trip	Number of samples	Sampling time	Sampling gear information
Gulf of St. Lawrence	DFO 2017 Gulf Longline Survey	76	Fall 2017	Hooked line
Gulf of St. Lawrence	DFO Teleost-14 Trawl Survey	83	August 2017	Trawl
Gulf of Maine/Georges Bank (NAFO 5Y and 5Ze)	The Nature Conservancy-Cape Cod Industry samples	42	Summer – Winter 2017	Gillnet
Gulf of Maine	Maine Department of Marine Resources – Gulf of Maine Survey	17	2018	Trawl
Newfoundland & Grand Banks	DFO Newfoundland Multi-species sSampling	17	Spring – Fall 2017	Hooked line
Newfoundland & Grand Banks	DFO 3Ps Halibut Port Sampling	45	November 2017	Line – hook sizes 15 – 16
Newfoundland & Grand Banks	Spanish Institute of Oceanography 3NO Survey	16	June 2018	-
Flemish Cap	Spanish Institute of Oceanography Flemish Cap Survey	11	Summer 2018	-

Newfoundland & Grand Banks, Scotian Shelf, Gulf of St. Lawrence (Cabot Strait)	DFO 2018 Longline Survey	461	Spring – Summer 2018	Hooked line

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Table 2. Number of SNPs, RAD loci, and strand mapping rates for SNP panels with different levels of filtering applied.

SNP panel and filtering step	SNPs	RAD loci	Proportion SNPs mapped to positive strand
Stacks exported	175,318	43,840	0.499
Filtered for minimum 90% genotyping per SNP, minimum read depth 15	89,801	33,988	0.497
Filtered for HWE	86,043	33,076	0.498
Filtered for sex locus and putative inversion	62,213	29,592	0.498
Filtered for LD, sex locus and putative inversion	61,662	29,550	0.498
Filtered to single SNP per RAD locus, sex locus and putative inversion	29,913	29,913	0.498

Figure 1. Sampling locations of 734 Atlantic Halibut samples genotyped with RAD loci, coloured by sampling region.

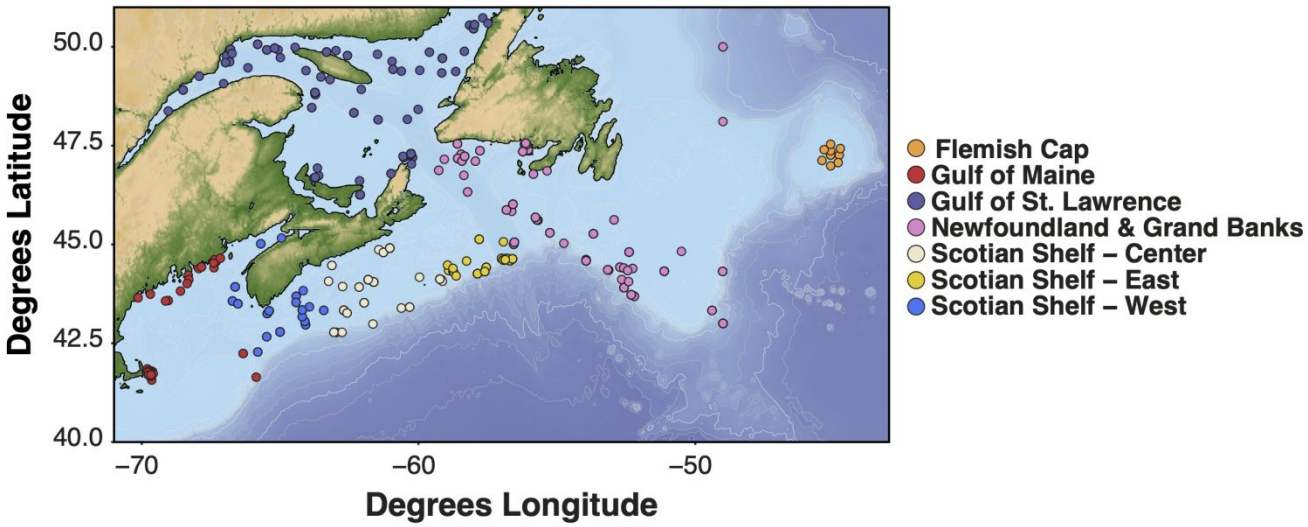


Figure 2. Principal component analysis (PCA) scores of 734 individuals on PC1 and PC3 axes from PCA on 86,043 SNPs, highlighting population structure (PC3) and a putative inversion (PC1) (A), q values of SNP association with K =3 retained PC axes, with outliers ($q < 0.05$) highlighted in red (thinned post-analysis to 15,000 randomly-thinned SNPs for visualization), (B), redundancy analysis of 734 individuals with principal components of temperature, dissolved oxygen and salinity (C), q values of SNP association with K =3 retained RDA axes, with outliers for each axis (q < 0.05, canonical score > 99.9th percentile) highlighted (thinned post-analysis to 15,000 randomly-thinned SNPs for visualization), (D)

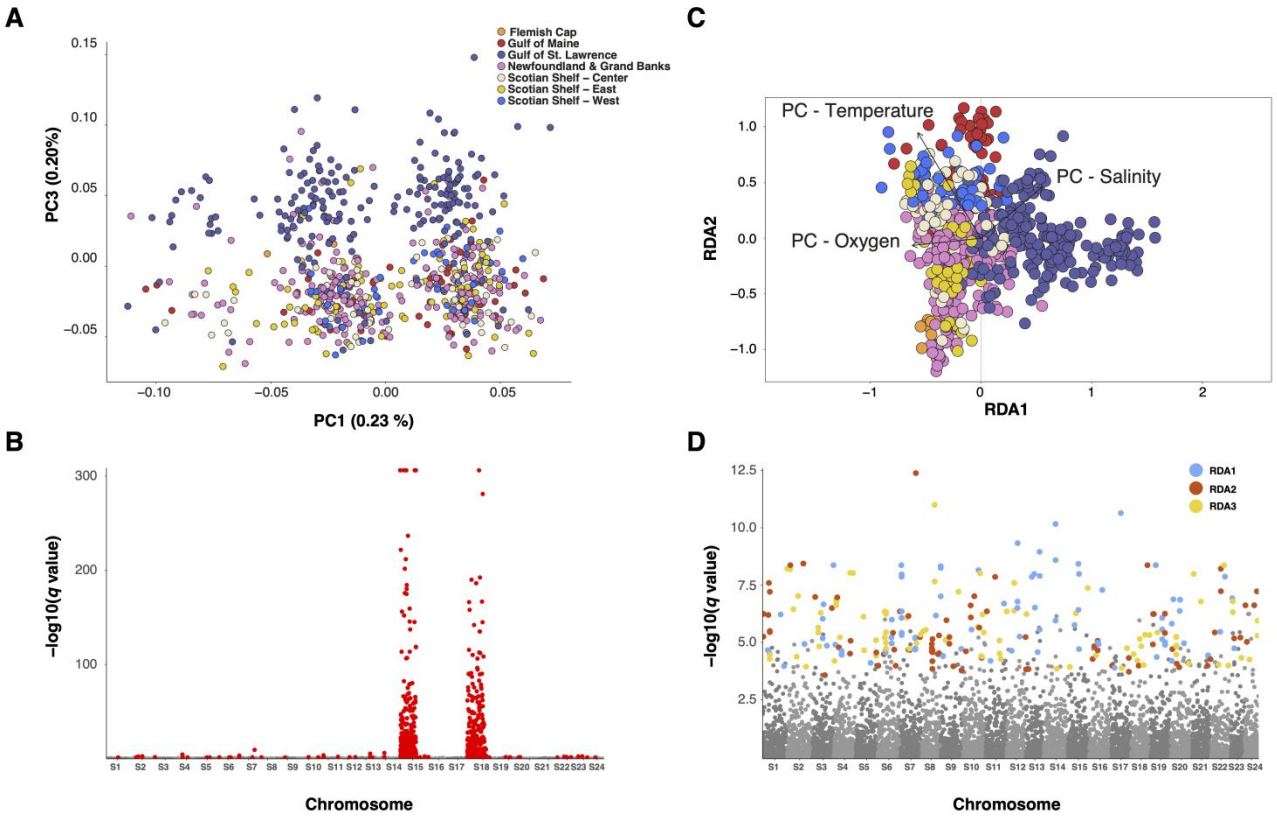


Figure 3. Distribution of F_{ST} between Gulf of St. Lawrence and Scotian Shelf and Grand Banks region (thinned post-analysis to 15,000 randomly-thinned SNPs for visualization), significant outliers with F_{ST} > 99th percentile above the significance line, and RDA outliers highlighted in red (A), admixture proportions from sparse non-negative matrix factorization (B) and PCA scores (C) of 734 individuals using 861 outlier SNPs.

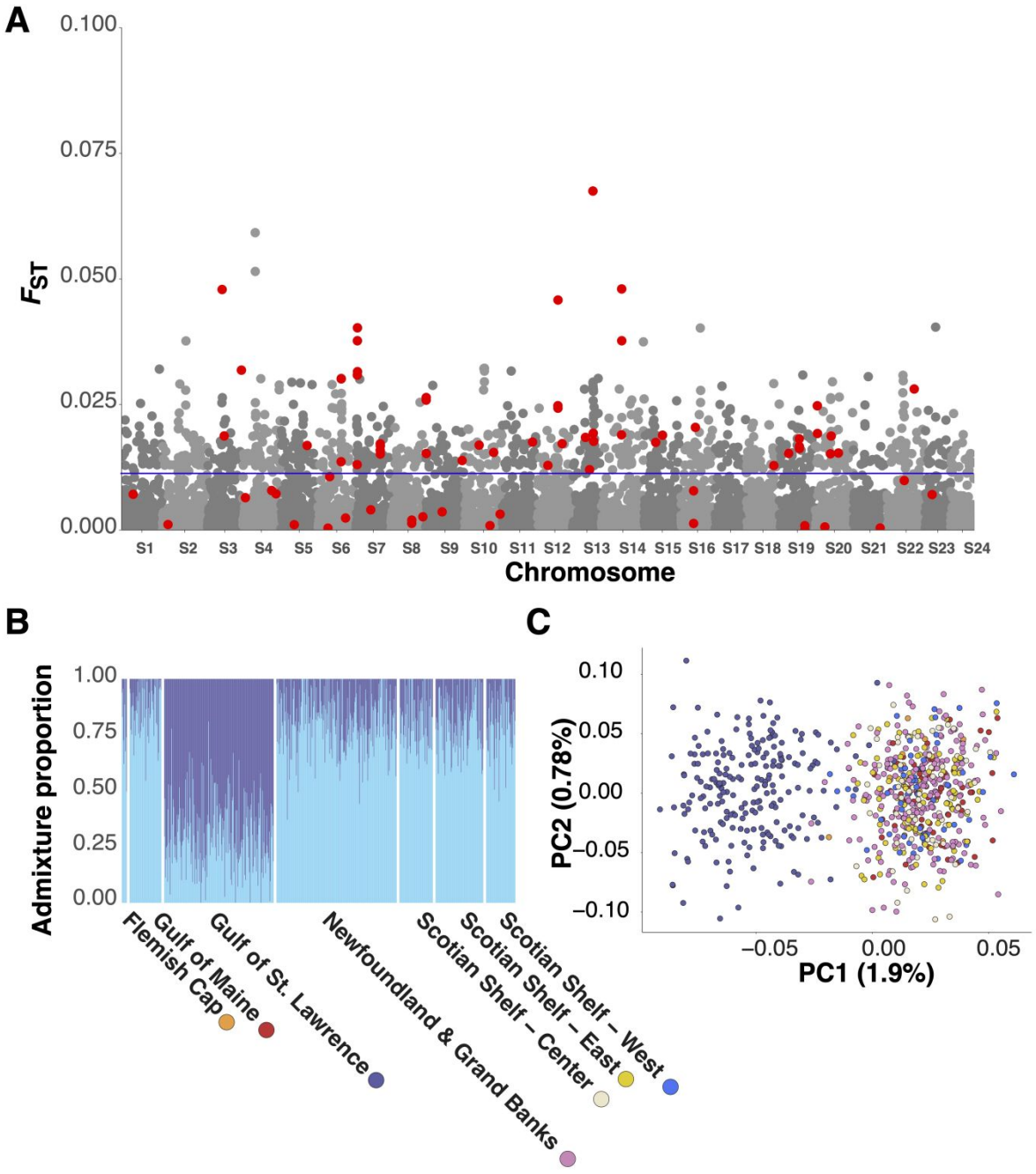


Figure 4. Percentage of each genotype of the chromosome 15 putative structural variant in each sampling region (A), F_{ST} between individuals homozygous for haplotype 1 and haplotype 2 across chromosome 15 (B), a heatmap of LD from 4 Mbp to 13 Mbp on chromosome S15 (C), and Pearson's r^2 correlation between LD (r^2) of each SNP with 50 adjacent SNPs and F_{ST} across chromosome 15, compared within the putative structural variant region (red) and outside of it (blue) (D).

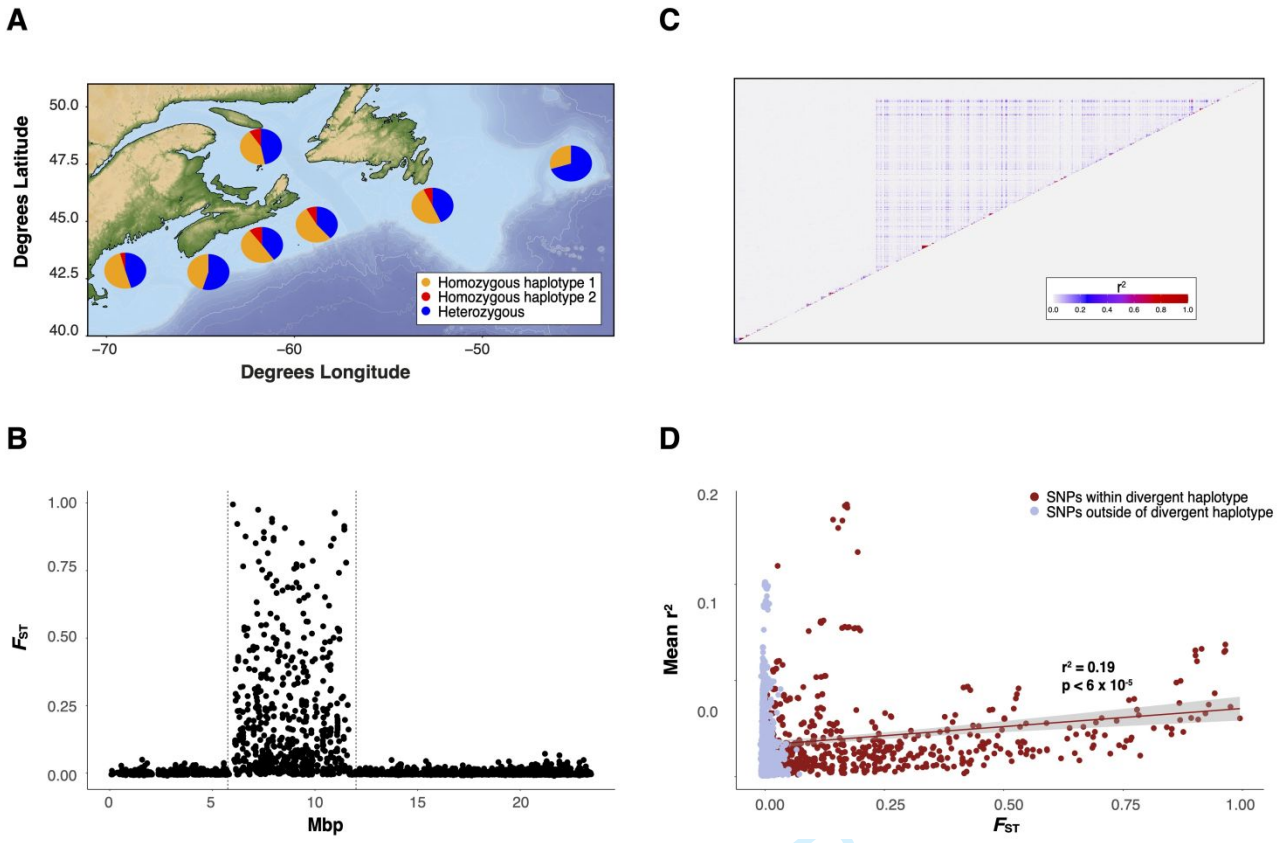
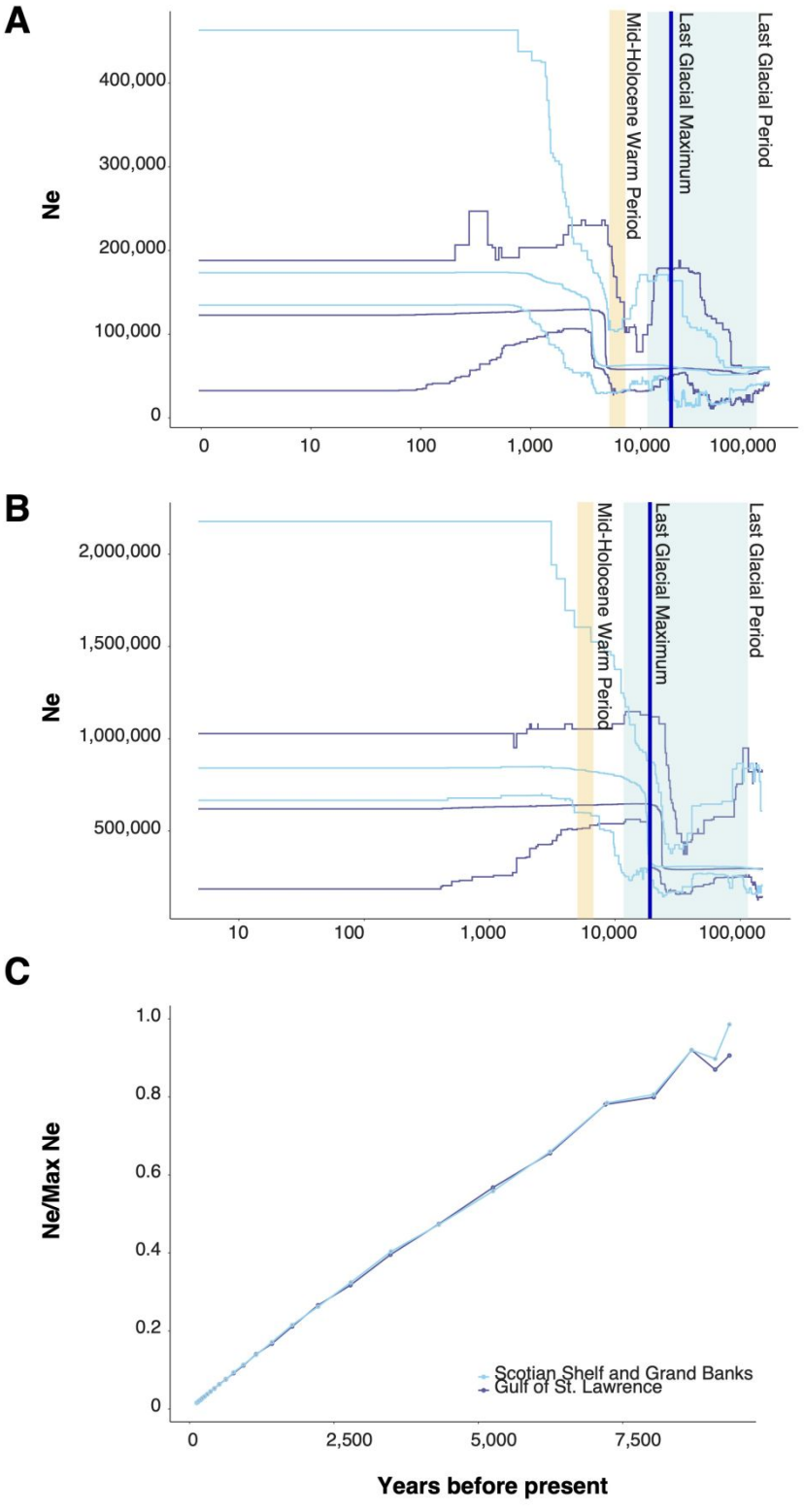
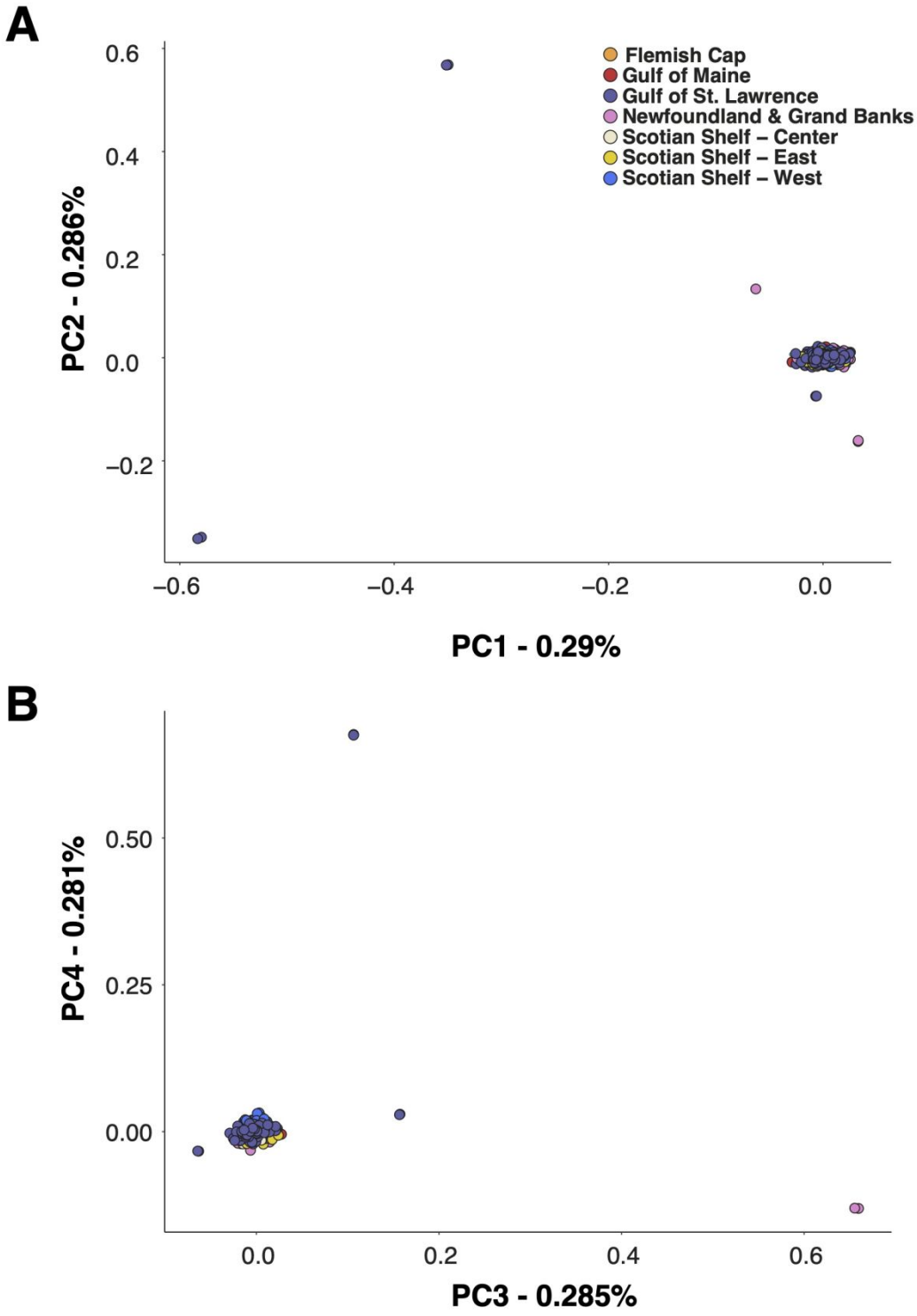


Figure 5. Reconstruction of effective population size (Ne) over time for Gulf of St. Lawrence and Atlantic continental shelf regions using *stairwayplot2* with mutation rate 1.0 x 10^{-8} (A) and mutation rate 2.0 x 10^{-9}, (B), and *SNeP* (C).



Supplementary Figure 1. Individuals demonstrating potential long-distance movement inferred from principal component analysis on all SNP loci (PCA), identified by scores on PC1, PC2 (A), PC3, and PC4.



640 **Supplementary Figure 2. PCA biplots for PCs of combined temperature, salinity, and oxygen**
641 **environmental variables.**
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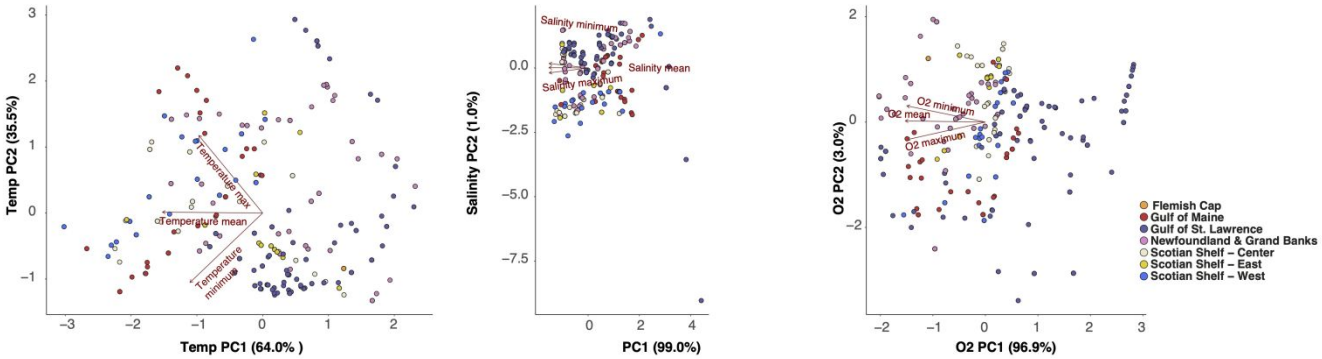

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644 **Supplementary Figure 3. Identification of a sex-associated region on chromosome 18 based on**
645 **principal component analysis (PCA) scores of 734 individuals on PC2 from PCA on 86,043 SNPs**
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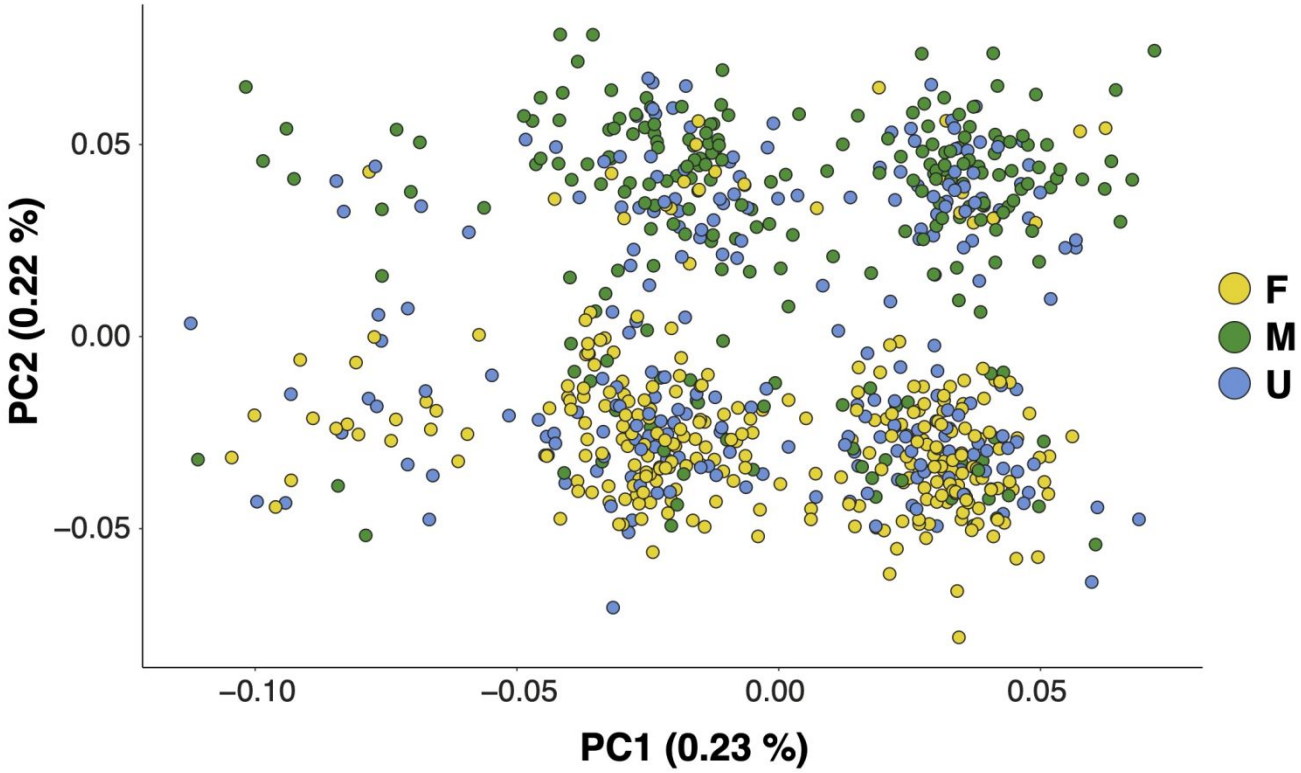
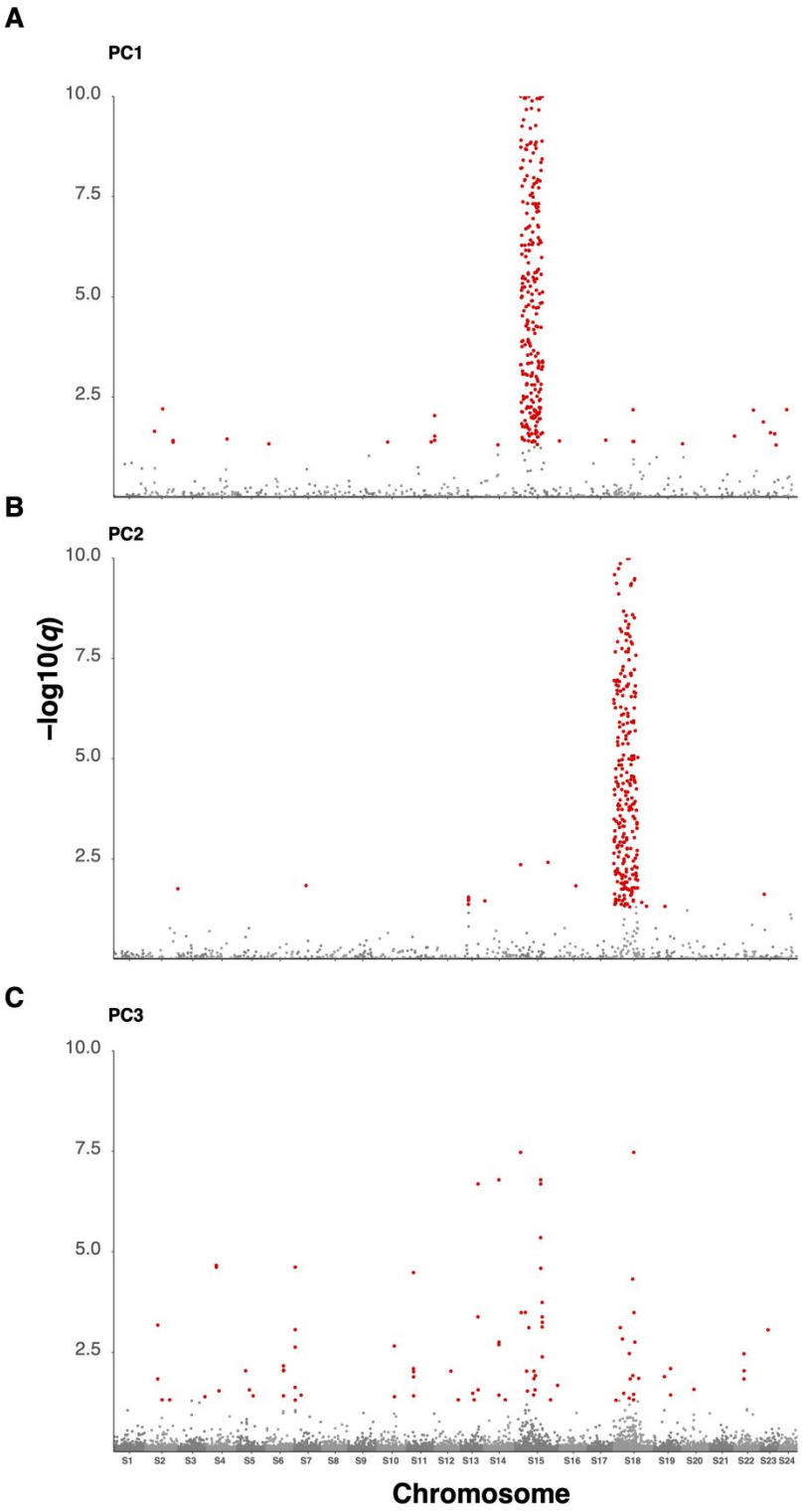
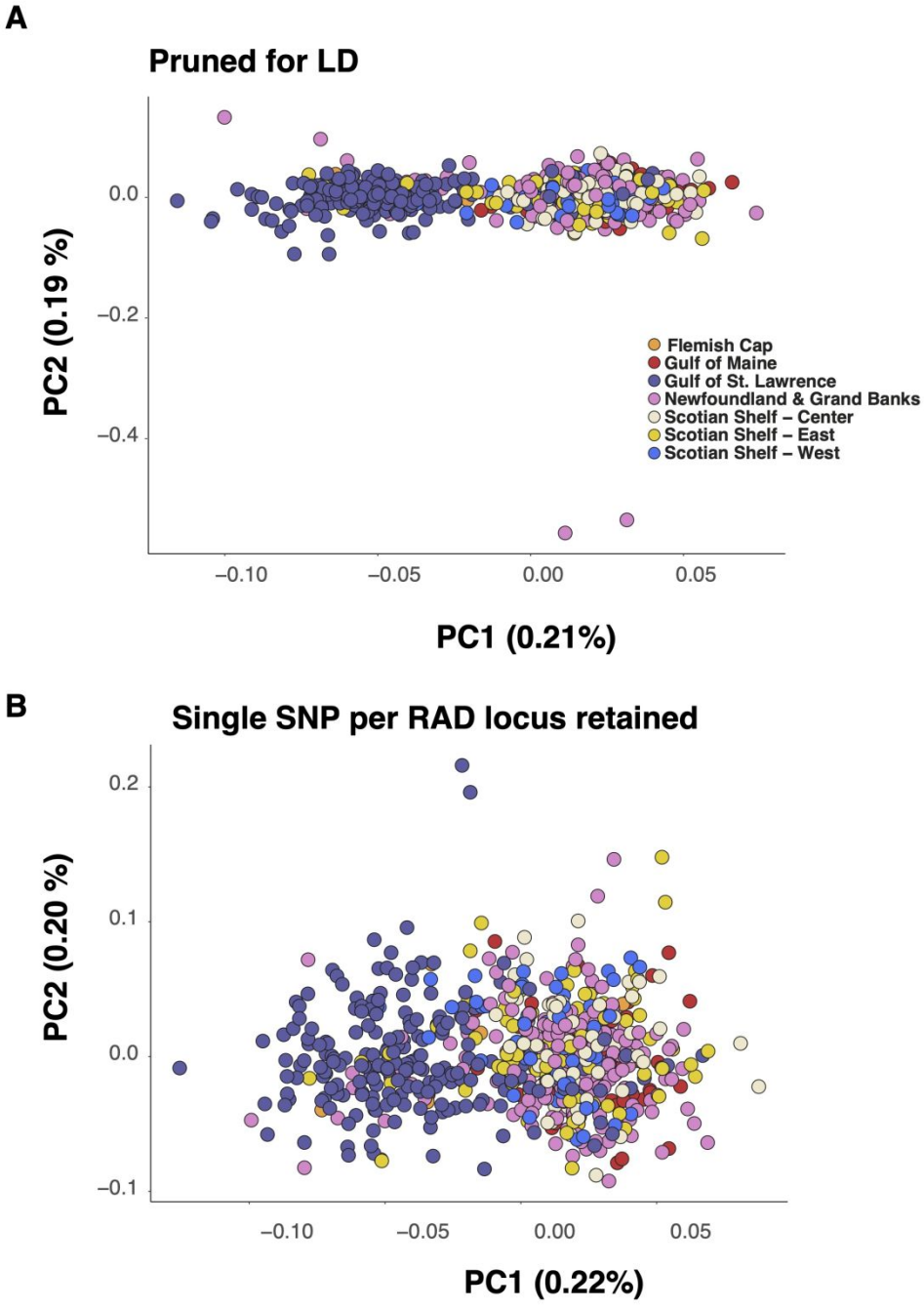


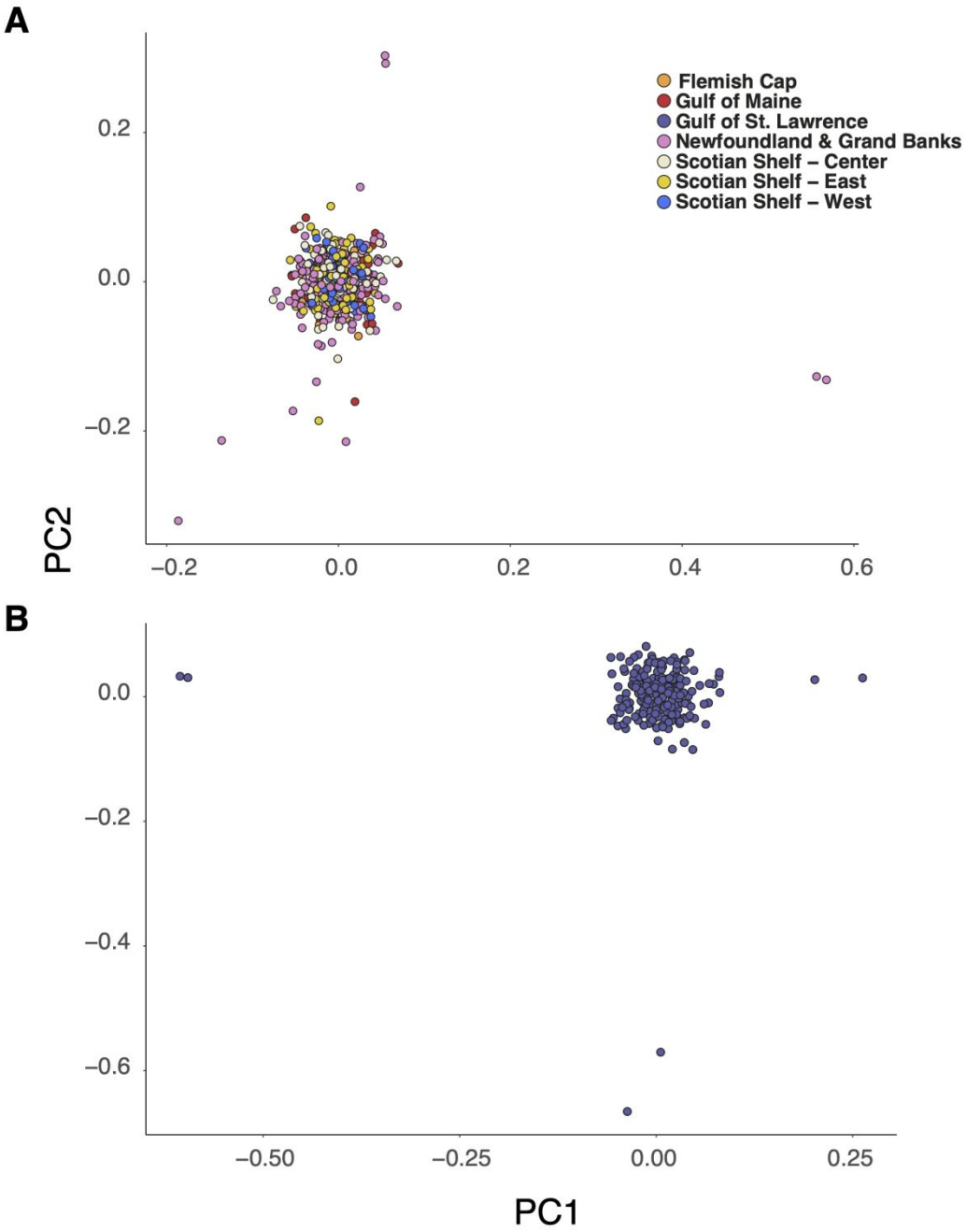
649 **Supplementary Figure 4. -log10(*q*) values of SNP association with PC1, A) PC2, B) and PC3 C),**
650 **with outliers ($q < 0.05$) highlighted in red (thinned post-analysis to 15,000 randomly-thinned**
651 **SNPs for visualization).**
652
653



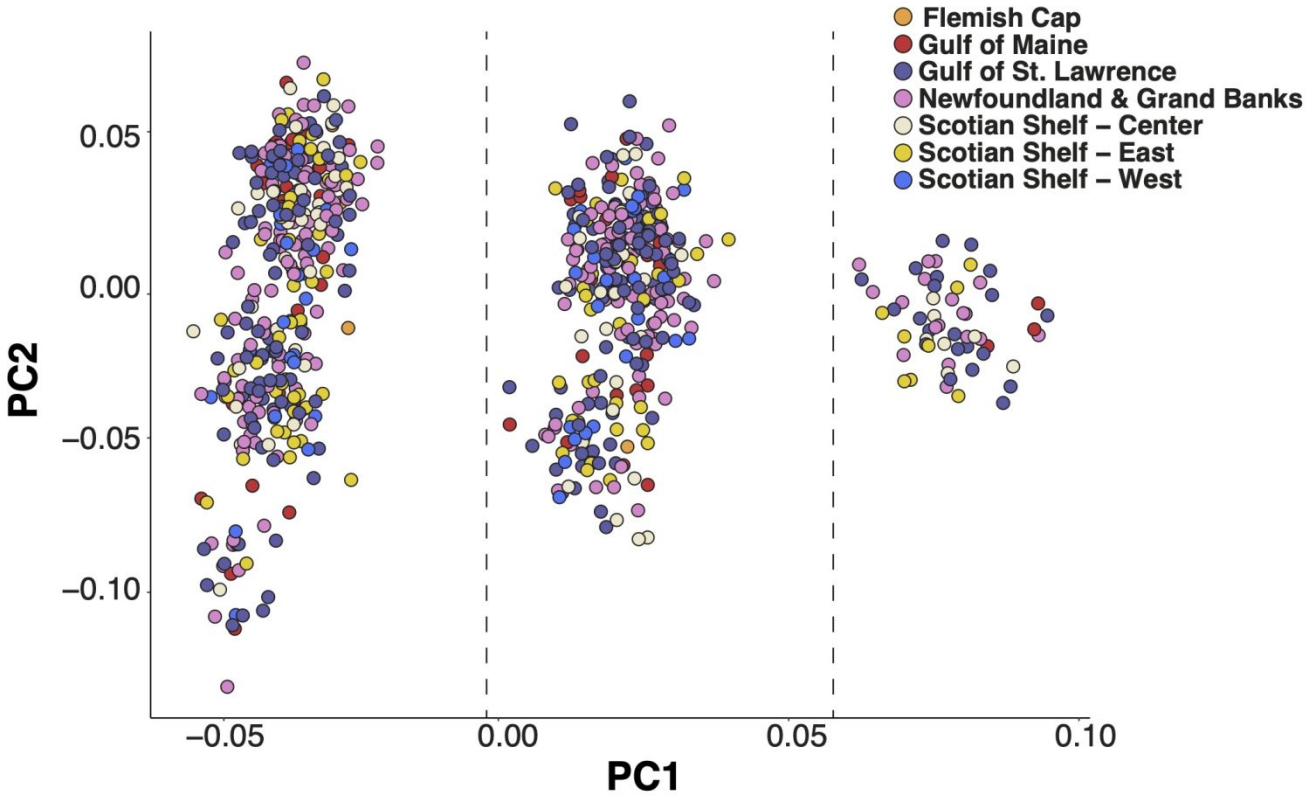
Supplementary Figure 5. Principal component analysis (PCA) scores of 734 individuals on PC1 and PC2 axes from PCA on 61,662 SNPs filtered for LD and with chromosomes S15 and S18 removed (A), and 29,913 SNPs filtered to retain a single SNP per RAD locus, and with chromosomes S15 and S18 removed.



Supplementary Figure 6. Principal component analysis (PCA) scores on PC1 and PC2 axes from PCA on 62,213 SNPs with chromosomes S15 and S18 removed on 523 individuals from the Atlantic continental shelf (A), and 211 individuals from the Gulf of St. Lawrence (B).



664 **Supplementary Figure 7. Chromosome 15 haplotype clusters inferred from chromosome 15 PCA**
665 **loadings on PC1.**



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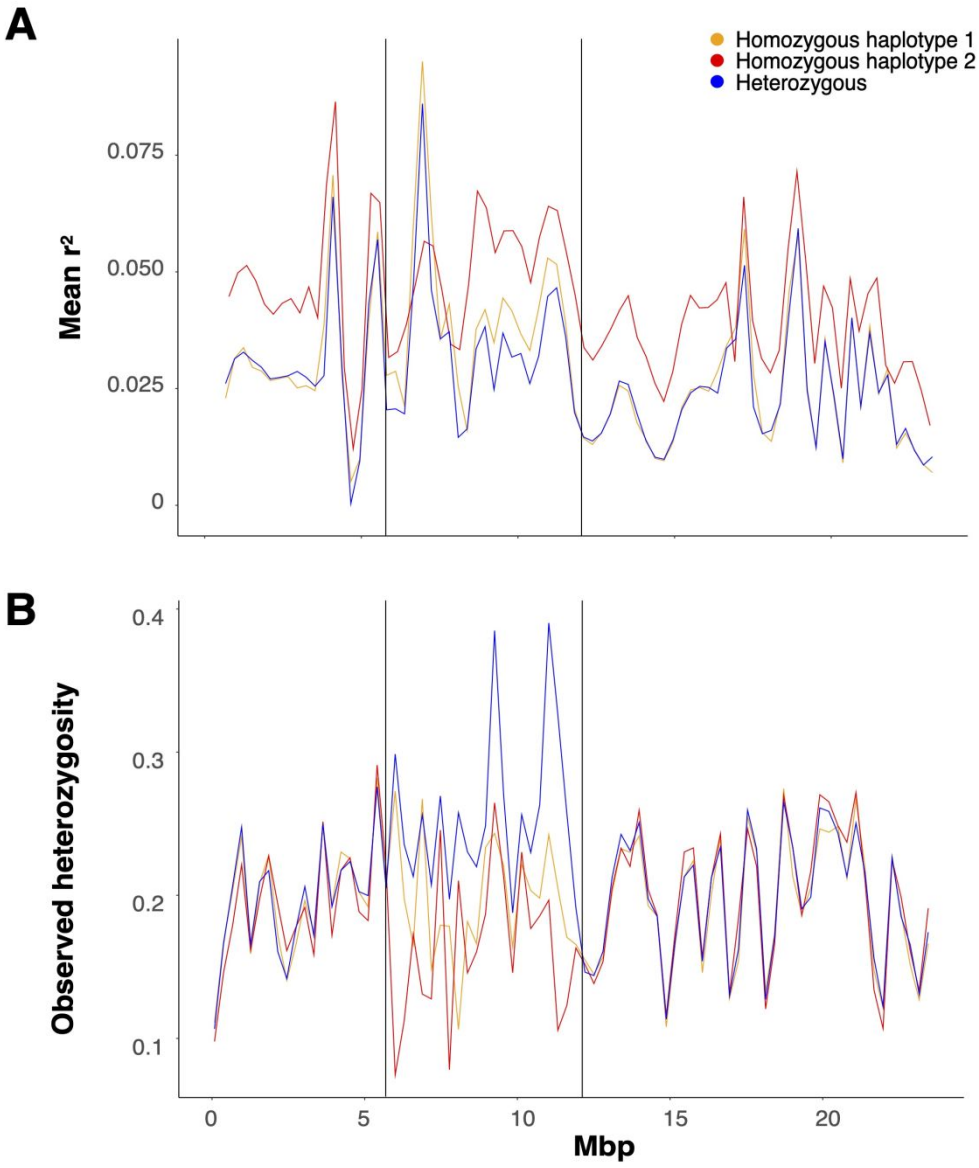
Supplementary Figure 8. Pearson's r^2 correlation between LD (r^2) of each SNP with 50 adjacent SNPs (A) and observed heterozygosity across chromosome S15, estimated separately within individuals homozygous or heterozygous for each haplotype.



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