Original Research

Oceanographic variation influences spatial genomic structure in the sea scallop, Placopecten magellanicus

Mallory Van Wyngaarden1 | Paul V. R. Snelgrove1,2 | Claudio DiBacco3 | Lorraine C. Hamilton4 | Naiara Rodríguez-Ezpeleta5 | Luyao Zhan6 | Robert G. Beiko6 | Ian R. Bradbury6,7

Abstract
Environmental factors can influence diversity and population structure in marine species and accurate understanding of this influence can both improve fisheries management and help predict responses to environmental change. We used 7163 SNPs derived from restriction site-associated DNA sequencing genotyped in 245 individuals of the economically important sea scallop, Placopecten magellanicus, to evaluate the correlations between oceanographic variation and a previously identified latitudinal genomic cline. Sea scallops span a broad latitudinal area (>10 degrees), and we hypothesized that climatic variation significantly drives clinal trends in allele frequency. Using a large environmental dataset, including temperature, salinity, chlorophyll a, and nutrient concentrations, we identified a suite of SNPs (285–621, depending on analysis and environmental dataset) potentially under selection through correlations with environmental variation. Principal components analysis of different outlier SNPs and environmental datasets revealed similar northern and southern clusters, with significant associations between the first axes of each ($R^2_{adj} = .66–.79$). Multivariate redundancy analysis of outlier SNPs and the environmental principal components indicated that environmental factors explained more than 32% of the variance. Similarly, multiple linear regressions and random-forest analysis identified winter average and minimum ocean temperatures as significant parameters in the link between genetic and environmental variation. This work indicates that oceanographic variation is associated with the observed genomic cline in this species and that seasonal periods of extreme cold may restrict gene flow along a latitudinal gradient in this marine benthic bivalve. Incorporating this finding into management may improve accuracy of management strategies and future predictions.

Keywords
adaptation, outlier loci, population genomics, RAD-seq, sea scallop, single-nucleotide polymorphism
1 | INTRODUCTION

The application of population genomic-based approaches to the study of marine population structure has revealed increasingly higher levels of genetic differentiation and population structure than previously recognized in multiple marine species (e.g., Benestan et al., 2015; Bradbury et al., 2013; Corander, Majander, Cheng, & Merila, 2013; Milano et al., 2014; Moura et al., 2014). Recent observations of fine-scale differentiation are changing our view of marine connectivity and marine population dynamics (Hauser & Carvalho, 2008). Limited dispersal may contribute to fine-scale population differentiation, but given large populations and large environmental gradients, selection may also contribute significantly to genetic differentiation among marine populations (Hauser & Carvalho, 2008). As such, studies supporting a role for selection in regulating marine connectivity continue to accumulate (Bradbury et al., 2010; Clarke, Munch, Thorrold, & Conover, 2010; Limborg et al., 2012; Milano et al., 2014; Sjöqvist, Godhe, Jonsson, Sundqvist, & Kremp, 2015; Van Wyngaarden et al., 2017). Researchers increasingly recognize the important role of selection in population connectivity, particularly for economically important species, because an accurate understanding of population structure and environmental influences can contribute to the identification of conservation units and allow prediction of a species’ response to climate change (Allendorf, Hohenlohe, & Luikart, 2010; Conover, Clarke, Munch, & Wagner, 2006; Sale et al., 2005).

Genomic studies increasingly highlight a role for selection in regulating marine population structure (Berg et al., 2015; Bradbury et al., 2010, 2014; Gagnaire et al., 2015; Gaither et al., 2015; Hellberg, 2009), and loci identified as putatively under selection repeatedly reflect small-scale genetic differentiation in multiple marine species (Bradbury et al., 2010; De Wit & Palumbi, 2013; Lamichhaney et al., 2012). Additionally, marine landscape genomic studies combining traditional landscape approaches with large genomic datasets have identified significant associations between climate and genetic structure (genetic–environmental associations) in numerous marine and anadromous species, including Atlantic herring (Clupea harengus) (Limborg et al., 2012), Atlantic cod (Gadus morhua) (Berg et al., 2015; Bradbury et al., 2010), purple sea urchin (Stronglylocentrotus purpuratus) (Pespeni & Palumbi, 2013), Atlantic salmon (Salmo salar) (Bradbury et al., 2014), European hake (Merluccius merluccius) (Milano et al., 2014), and Chinook salmon (Oncorhynchus tshawytscha) (Hecht, Matala, Hess, & Narum, 2015). Although historically most marine population genomic studies have focused on fish species, work on other taxa, including invertebrates, is increasing (e.g., Benestan et al., 2015; Pespeni & Palumbi, 2013). The pervasiveness of genetic–environmental associations across taxa and life histories supports the hypothesis that environmentally associated selection may structure marine populations.

The sea scallop, Placopecten magellanicus (Gmelin) (Figure 1), is an economically important benthic marine bivalve with a range that extends from North Carolina, USA, to Newfoundland, Canada (Posgay, 1957). The scallop fishery in both the United States and Canada extends back over 100 years and is one of the most economically important fisheries on the east coast of North America (DFO 2016; Naidu & Robert, 2006; NOAA 2016). The sessile adult scallops live in isolated beds up to 300 m deep and undergo broadcast spawning. Juvenile scallops have a planktonic period of development of up to 40 days, which is conducive with the potential for long distance dispersal among populations (Davies, Gentleman, DiBacco, & Johnson, 2014; Tian et al., 2009). The scallop range spans a vast latitudinal area where the cold Labrador Current meets the warm Gulf Stream. This convergence leads to large gradients in ocean temperature and other environmental factors experienced by different scallop populations, all of which may be influenced by the oceanographic properties of major currents and storm-related mixing along coastal areas (Townsend, Thomas, Mayer, Thomas, & Quinlan, 2006). Several oceanographic barriers (such as current fronts) along the range may also influence larval dispersal and survival among populations (Townsend et al., 2006).

Previous genetic work on the sea scallop detected significant but weak population structure among geographic locations off eastern North America using microsatellites, AFLPs, and SNPs (Kenchington, Patwary, Zouros, & Bird, 2006; Owen & Rawson, 2013; Van Wyngaarden et al., 2017). Recently, Van Wyngaarden et al. (2017) resolved significant spatial structuring in sea scallops visible primarily in outlier loci detected using F_{ST}-based outlier detection methods and genomewide RAD-seq (restriction site-associated DNA sequencing)-derived SNPs. Taken together, the results from these studies suggest that both limited dispersal and selection associated with local adaptation across the species range may spatially structure scallop populations, despite high potential for gene flow. Here, we build directly on existing studies and known population structure to identify environmental variables that may contribute to non-neutral divergence among sea scallop populations. Using the RAD-seq-derived SNPs identified in Van Wyngaarden et al. (2017), we focus solely on environmental association-based outlier tests to identify loci potentially under selection and directly compare our results to existing work and the results of previous outlier tests.

Considering the unique oceanographic features, the large latitudinal range, and previously identified clinal population structure along the range of the sea scallop, we hypothesize that directional selection...
and local adaptation drive sea scallop population structure and that ocean temperature likely contributes significantly to adaptation of the species to its local environment. Our specific objectives are to: (1) explore spatial variation in environmental variables across the range of the sea scallop, (2) use environmental correlation-based outlier detection methods to pinpoint potential targets of environment-based selection across the genome of the sea scallop, and (3) identify potentially important environmental drivers of population structure and adaptation in scallops. This work represents one of the first population genomic studies on a bivalve species or on an invertebrate species with a planktonic juvenile and sessile adult life stage. This work also incorporates both environmental association-based outlier detection and nonlinear random-forest analysis (Breiman, 2001), a machine-learning strategy only recently applied to genomic analysis that can help to account for interaction and covariation between variables (Brieuc, Ono, Drinan, & Naish, 2015). We extend a previous study identifying latitudinal clinal trends in allele frequency across the range using 7163 RAD-seq-derived SNPs (Van Wyngaarden et al., 2017) and identify environmental associations and possible mechanisms.

2 METHODS

2.1 Sample collection and RAD-seq

Sample collection and RAD-seq follow Van Wyngaarden et al. (2017). Using divers or bottom trawls, 252 adult scallops were collected from a total of 12 locations across the entire range of the species between 2011 and 2013 (Table 1, Figure 2). A minimum of 12 scallops were collected per population (mean ± SD, 20.4 ± 2.8 scallops).

Muscle tissue samples were collected and preserved in AllProtect (Qiagen) or 80% ethanol. DNA extraction and RAD-seq library preparation were performed at the Aquatic Biotechnology Lab, Bedford Institute of Oceanography in Dartmouth, Nova Scotia. RAD-seq libraries were prepared using SbfI as described by Etter, Preston, Bassham, Cresko, and Johnson (2011) (see also Etter, Bassham, Hohenlohe, Johnson, and Cresko, 2011) with modifications. Each library was created using 22 individuals from each sampling location (or 20 individuals for SUN) with a unique in-line barcode in the P1 adapter for each individual. The P1 adapter barcodes were 6 bp in length for all populations except for SSB, GEO, and SUN where the barcodes ranged from 5 bp to 9 bp. The barcodes for SSB, GEO, and SUN were chosen to ensure equal distribution of all nucleotides at each base position and to maximize the edit distance (Faircloth & Glenn, 2012). Based on edit tags analysis (Faircloth & Glenn, 2012), the variable length barcodes edit distance ranged from two to eight (modal edit distance was six). Sequencing was performed

| Site name          | Site code | Latitude | Longitude | Number of scallops used in analysis |
|--------------------|-----------|----------|-----------|-------------------------------------|
| Sunnyside, NL      | SUN       | 47.82    | −53.87    | 20                                  |
| Little Bay, NL     | LTB       | 47.15    | −55.10    | 21                                  |
| Magdalen Islands   | MGD       | 47.11    | −62.02    | 21                                  |
| Northumberland Strait | NTS  | 46.13    | −63.77    | 22                                  |
| Passamaquoddy Bay  | PSB       | 45.06    | −67.02    | 12                                  |
| Bay of Fundy       | BOF       | 44.68    | −66.07    | 22                                  |
| Scotian Shelf - Middle | SSM  | 44.52    | −60.64    | 19                                  |
| Gulf of Maine Inshore | GMI   | 44.52    | −67.03    | 20                                  |
| Browns Bank        | SSB       | 42.84    | −66.14    | 22                                  |
| Gulf of Maine Offshore | GMO | 42.44    | −70.39    | 22                                  |
| George’s Bank      | GEO       | 41.61    | −66.36    | 22                                  |
| Mid Atlantic Bight | MDA       | 38.82    | −73.60    | 22                                  |

*Several neighbouring sites sampled as one location*
at the McGill University and Génome Québec Innovation Centre, Montréal, Canada, using a HiSeq 2000 (Illumina) as 100-bp paired-end sequences with one library per lane.

SNPs were detected using the de novo pipeline in STACKS v0.9999 (Catchen, Amores, Hohenlohe, Cresko, & Postlethwait, 2011). Loci were assembled using ustacks, requiring a minimum depth of coverage for a stack (m) of five and allowing four maximum nucleotide mismatches (M) between stacks. The catalog of loci was assembled using cstacks allowing a distance between loci in the catalog (n) of six. The final dataset was filtered using PLINK v1.07 (Purcell, 2009; Purcell et al., 2007) to include only RADtags present in 75% of individuals in SNP discovery and calling; all SNPs included in the analysis were present in 75% of individuals with a minor allele frequency (MAF) greater than 5%. Furthermore, we excluded individuals with more than 20% missing loci from the analysis. Loci were filtered for Hardy–Weinberg equilibrium using the program GENEPOP v.4 (Rousset, 2008), excluding loci out of equilibrium in six or more populations from the analysis (<0.7% of all loci).

2.2 | Environmental data collection and processing

We amalgamated environmental data from several databases; from Fisheries and Oceans Canada: Climate (Gregory, 2004) (years 1970–2013), BioChem (Devine et al., 2014; DFO 2014) (years 2009–2014), and AZMP (DFO 2015), and from the National Oceanographic and Atmospheric Administration in the United States of America (NOAA, years 1990–2010), and the MODIS satellite database (NASA Goddard Space Flight Center Ocean Ecology Laboratory 2014) (years 2002–2013). Data were averaged over multiple years available to remove the signatures of short-term variation in the marine environment. Available physical and chemical variables included water temperature, salinity, sigma-t (a measure of water density related to temperature and salinity), chlorophyll a (a measure of primary productivity), and concentrations of SiO\textsubscript{4}, NO\textsubscript{3}, NO\textsubscript{2}, and PO\textsubscript{4} (nutrients required for many marine primary producers).

We averaged data from all data sources within a bounding box of one square degree around each sample site to create site-specific averages for each data type used in the analysis. Data were separated into surface and depth values based on the collection depth for each sampling location. Surface values encompassed depths between zero and 20 m except for collection sites less than 20 m depth, where 10 m was used as the surface cutoff. We averaged values from a cutoff approximately 10 m above a given collection depth to the collection depth for depth-profiled variables. In cases where multiple sample collection depths were provided, depth cutoff parameters were altered to include the entire range of collection depths (Table 2).

Data validation and preparation were completed using R (R Core Development Team 2012). To address natural seasonal variation in the data, we calculated z-scores for each variable for each sample site per month and removed outliers where necessary. Variables with missing data for more than six sites were removed from subsequent analyses (29 variables in total were removed). For the remaining variables with missing data, we used single imputation using neighboring sites to estimate missing values (sites arranged by latitude, averaging sites directly north and south of the missing site). Following outlier removal and imputation, we standardized data to zero mean and unit variance by subtracting the mean and dividing by the standard deviation. We then identified site-specific maximum and minimum values as well as seasonal averages for each variable, basing seasons largely on equinoxes. Winter included January, February, and March; spring included April, May, and June; summer included July, August, and September; and autumn included October, November, and December. The final dataset contained 90 variables spanning all available data types (hereby referred to as AllEnv). The 29 variables removed due to missing data translated to only six removed final variables following

| Site name          | Site code | Depth range (m) | Surface range (m) | Bounding box top left | Bounding box bottom right |
|--------------------|-----------|----------------|-------------------|-----------------------|--------------------------|
| Sunnyside, NL      | SUN       | −10 to −20     | 0 to −10          | 48.82                 | 54.87                    |
| Llittle Bay, NL    | LTB       | −30 to −40     | 0 to −20          | 48.15                 | 56.10                    |
| Magdalen Islands   | MGD       | −35 to −45     | 0 to −20          | 48.11                 | 63.02                    |
| Northumberland Strait | NTS   | −15 to −25     | 0 to −10          | 47.13                 | 64.77                    |
| Passamaquoddy Bay  | PSB       | −20 to −30     | 0 to −20          | 46.06                 | 68.02                    |
| Bay of Fundy       | BOF       | −30 to −140    | 0 to −20          | 45.68                 | 67.07                    |
| Scotian Shelf - Middle | SSM     | −35 to −50     | 0 to −20          | 45.52                 | 61.64                    |
| Gulf of Maine Inshore | GMI     | −60 to −80     | 0 to −20          | 45.52                 | 68.03                    |
| Browns Bank        | SSB       | −50 to −125    | 0 to −20          | 43.84                 | 74.14                    |
| Gulf of Maine Offshore | GNO   | −70 to −90     | 0 to −20          | 43.44                 | 71.39                    |
| George's Bank      | GEO       | −50 to −100    | 0 to −20          | 42.61                 | 67.36                    |
| Mid Atlantic Bight | MDA       | −70 to −90     | 0 to −20          | 39.82                 | 74.60                    |

| Site name          | Site code | Depth range (m) | Surface range (m) | Bounding box top left | Bounding box bottom right |
|--------------------|-----------|----------------|-------------------|-----------------------|--------------------------|
| Sunnyside, NL      | SUN       | −10 to −20     | 0 to −10          | 48.82                 | 54.87                    |
| Llittle Bay, NL    | LTB       | −30 to −40     | 0 to −20          | 48.15                 | 56.10                    |
| Magdalen Islands   | MGD       | −35 to −45     | 0 to −20          | 48.11                 | 63.02                    |
| Northumberland Strait | NTS   | −15 to −25     | 0 to −10          | 47.13                 | 64.77                    |
| Passamaquoddy Bay  | PSB       | −20 to −30     | 0 to −20          | 46.06                 | 68.02                    |
| Bay of Fundy       | BOF       | −30 to −140    | 0 to −20          | 45.68                 | 67.07                    |
| Scotian Shelf - Middle | SSM     | −35 to −50     | 0 to −20          | 45.52                 | 61.64                    |
| Gulf of Maine Inshore | GMI     | −60 to −80     | 0 to −20          | 45.52                 | 68.03                    |
| Browns Bank        | SSB       | −50 to −125    | 0 to −20          | 43.84                 | 74.14                    |
| Gulf of Maine Offshore | GNO   | −70 to −90     | 0 to −20          | 43.44                 | 71.39                    |
| George's Bank      | GEO       | −50 to −100    | 0 to −20          | 42.61                 | 67.36                    |
| Mid Atlantic Bight | MDA       | −70 to −90     | 0 to −20          | 39.82                 | 74.60                    |
minimum, maximum, and seasonal calculations. We repeated all analyses using only the temperature, salinity, and chlorophyll a variables (n = 36 variables, henceforth CST), given that we expected these to be the most likely to associate with selection; they not only provide evidence of food availability but also characterize water mass properties that can affect all trophic levels.

2.3 Detection of outlier loci

We used two separate methods to detect outlier loci using both environmental datasets (four tests in total). The first method used a Bayesian framework implemented in the program BAYENV2 (Coop, Witonsky, Di Rienzo, & Pritchard, 2010; Guenther & Coop, 2013). This method calculates a set of "standardized allele frequencies" that controls for population history and structure when detecting loci whose allele frequencies show significant associations with environmental variation. This method then calculates a Bayes factor (BF), which measures the weight of evidence for a model in which the environmental variable affects the allele frequency of a locus versus a null model with no environmental variable effect. To calculate the "standardized allele frequencies," we randomly selected 700 loci (9.8% of total loci) identified as neutral (not under selection) in Van Wyngaarden et al. (2017). The null model correlation matrix was estimated from these loci in three repetitions of 100,000 iterations. We visually compared correlation matrices from the final iterations of each run to each other and to an $F_{ST}$ matrix of the neutral loci and determined there were no differences in the major patterns of the matrices. The final matrix from the first run was selected as the neutral matrix for use in further analysis. The final analysis detected locus-specific deviations from the "standardized allele frequencies" using 100,000 iterations. BFs were calculated at every locus for each environmental variable separately. To assess the significance of each BF (and the likelihood of classifying a locus as an outlier), we created five bins of loci based on the global minor allele frequency, as recommended in Coop et al. (2010) and implemented in Hancock et al. (2010) (Table 3). We selected loci with BFs in the top 5% of the range of BFs for each bin as outliers.

Latent factor mixed models (LFMMs) as described in Frichot, Schoville, Bouchard, and François (2013) were implemented as the second method of outlier detection in the R package LEA (Frichot & François, 2015). This method uses latent factors in a linear mixed model to control for population structure (the number of genetic clusters within a group of populations, K) while detecting correlations between environmental and genetic variation. Previous analysis using the program STRUCTURE v.2.2.4 (Pritchard, Stephens, & Donnelly, 2000) detected two genetic clusters (K = 2) (Van Wyngaarden et al., 2017), and the genomic inflation factor analysis (GIF) in LEA corroborated this result. The models were run for three repetitions, with a burn-in of 5,000 followed by 15,000 iterations. We combined Z-scores from the three repetitions using the median, calculated adjusted p-values to correct for multiple testing, and produced a list of candidate outlier loci for each environmental variable (FDR = 0.05) following Frichot, Schoville, de Villemereuil, Gaggiotti, and Francois (2015). To ensure we included any loci potentially under selection, for AllEnv and CST separately, we combined the list of detected outliers from both BAYENV2 and LFMM to create two final outlier lists (AllEnvOutlier for AllEnv and CSTOutlier for CST).

2.4 Environmental factors that influence genetic variation

We conducted principal components analysis (PCA) using the AllEnvOutlier and CSTOutlier loci using the R package adegenet (Jombart, 2008) to examine population structure among the sampled populations at outlier loci. To examine the relationship between environmental and genetic variation among our collection sites, we calculated population-specific allele frequencies for AllEnvOutlier and CSTOutlier using the R package gstudio (Dyer, 2014). Next, we ran PCA on population-specific allele frequencies for AllEnvOutlier and CSTOutlier (AllEnvOutlierPCA and CSTOutlierPCA), and the population-specific environmental data in AllEnv and CST (AllEnvPCA and CSTPCA) using the R package adegenet. Linear regression was then performed between the first principal component (PC) from AllEnvOutlierPCA (AllEnvOutlierPCA1) and the first PC from the PCA on AllEnv (AllEnvPC1) as well as the first PC from CSTOutlierPCA (CSTOutlierPC1) and the first PC from the PCA on CST (CSTPC1).

We then conducted redundancy analysis (RDA), a multivariate canonical correlation analysis, using the R package vegan (Oksanen et al., 2015) on population-specific allele frequencies for AllEnvOutlier and CSTOutlier and selected PCs from AllEnvPCA and CSTPCA, respectively. This analysis allowed us to determine which environmental variables used as explanatory variables in the RDA best explain the genetic population structure. Each PC that explained more than 5% of the total explainable variance in the AllEnvPCA (five axes) and CSTPCA (four axes) was selected as an explanatory variable. Backward stepwise variable selection using 1,000 or 10,000 iterations selected the most valuable environmental PCs within the model. To determine the proportion of model variation attributable to climate versus geographic distance between populations versus combined effects, we next performed partial RDA (pRDA), conditioning the genetic matrix on the distances from the furthest north population (SUN) along a one-dimensional transect that included all populations [estimated using GOOGLE EARTH (2013)].

| Bin | Number of loci | MAF range |
|-----|----------------|-----------|
| A   | 3,566          | 0.05–0.139|
| B   | 1,390          | 0.14–0.229|
| C   | 908            | 0.23–0.319|
| D   | 691            | 0.32–0.409|
| E   | 608            | 0.41–0.5  |
Multiple linear regressions quantified the direction and magnitude of the effect of environmental variables on genetic variation. We used results from RDA to select environmental variables used in the analyses. After examining weightings of the environmental variables on the important PCs selected during RDA, we selected the five most highly weighted variables from each PC for use as explanatory variables in linear mixed models. Based on results from the initial linear mixed models (see Section 3), we generated models focusing on measurements of water temperature at surface and at depth (Table 4). For each response variable (AllEnvOutlierPC1 and CSTOutlierPC1), we fitted a global multiple regression model with all selected environmental variables. We then used the R package MuMIn (Barton, 2014) to run all possible configurations of the global model and pinpointed the best model fits with AICc model selection. We examined cumulative AICc model weights to rank each parameter in order of importance and estimated coefficients for each environmental parameter using model averaging (Arnold, 2010).

We also used nonlinear random-forest (RF) analysis to identify important environmental variables and then compared key drivers with those identified using multiple linear regressions. One key attribute of RF is the automatic computation of variable importance, which allows us to determine which environmental variables influence population structure. Additionally, RF considers interaction between predictor variables and may manage the covariation among environmental variables more effectively than the multiple linear regression approach (Brieuc et al., 2015). This ensemble approach benefits from growing a large group of decision trees to improve overall performance.

RF cannot tolerate missing data, so we used a method based on weighted k nearest neighbors (KNN) called KNNcatImpute (Schwender, 2012) to impute the missing genotypes in our genetic SNP data using the scrime package in R (Schwender & Fritsch, 2013). After imputation, the individual genotypes at each outlier SNP were transformed to categorical data. SNPs are a biallelic genetic marker and only two alleles and three types of genotypes can be present at each SNP; the built RF is thus a three-class classification model. Environmental variables were used as predictors of individual genotypes at each outlier SNP using 1,001 trees.

We used permutation importance, the variable importance function built in RF, to rank the relative roles of environmental variables in influencing population structure. To obtain a reliable estimation of variable importance, we applied 10-fold cross-validation, dividing the entire dataset into 10 subsets. Nine subsets trained the RF model, and the other subset was used for validation; this process was repeated 10 times for each SNP genotype. In each of the 10 runs, we calculated a permutation importance array for all environmental variables. Noting

| Method of variable selection | Variables included                                                                 | Response variable |
|------------------------------|-----------------------------------------------------------------------------------|-------------------|
| Most highly weighted variables from AllEnvPCs selected by RDA | Deep average autumn salinity  
Deep minimum SiO$_4$  
Surface average autumn salinity  
Surface average winter temperature  
Surface minimum temperature | AllEnvOutlierPC1 |
| Most highly weighted variables from CSTPCs selected by RDA | Deep average winter temperature  
Deep average minimum temperature  
Surface average winter temperature  
Deep maximum salinity  
Deep average autumn salinity  
Surface maximum chlorophyll A  
Surface average spring chlorophyll A  
Surface average summer chlorophyll A  
Deep minimum chlorophyll A  
Surface minimum chlorophyll A | CSTOutlierPC1 |
| Temperature variables selected following the results of initial linear mixed models | Deep average autumn temperature  
Deep average spring temperature  
Deep average summer temperature  
Deep average winter temperature  
Surface average autumn temperature  
Surface average spring temperature  
Surface average summer temperature  
Surface average winter temperature | AllEnvOutlierPC1, CSTOutlierPC1 |
that importance values can be negative, we computed the exponential values of the importance array and averaged each importance value over the total importance sum of all environmental variables to generate an importance proportion array. The importance proportions were averaged over the 10 runs to determine average importance proportions. For each SNP genotype output (621 for AllEnv and 285 for CST), an RF model was built to calculate an array of permutation importance proportions for all environmental variables. We calculated the overall average importance proportion for each environmental variable over all loci. All RF analyses were performed using RandomForest package in R (Liaw & Wiener, 2002).

2.5 | Gene ontology

We performed gene ontology (GO) analysis on AllEnvOutlier and CSTOutlier in the program Blast2GO (Conesa et al., 2005) using the program default parameters and InterProScan to improve GO annotation quality.

3 | RESULTS

3.1 | Sample collection and RAD-seq

Following filtering and quality control steps, we included 245 individual scallop samples in our analysis (97.2% of sequenced individuals), 19672 RADtags (14.9% of initial RADtags), and 7216 SNPs (4.2% of initial SNPs) (Table 5). Read count per individual per RADtag averaged 56.12 ± 46.64 (mean ± SD). Final filtering required SNPs to be present in >95% of individuals with a MAF >5% and removed individuals with >20% missing data. The final dataset included 245 individual scallop samples and 7163 SNPs. Average pairwise \( r^2 \) values indicating linkage disequilibrium remained low overall (0.0044 ± 0.0098, mean ± SD) (Van Wyngaarden et al., 2017).

| TABLE 5 | Number of Placopecten magellanicus individuals and number of SNP loci included in initial RAD-sequencing and final analysis following quality control |
| Parameter | Value |
|---|---|
| Individuals sequenced | 252 |
| Individuals following QC | 245 (97.2% of Individuals sequenced) |
| Initial RAD tags | 131,897 |
| RAD tags following QC | 19,672 (14.9% of initial RAD tags) |
| Initial SNPs | 173,482 |
| SNPs following QC | 7,216 (4.2% of Initial SNPs) |
| SNPs in HWE | 7,163 (99.3% of SNPs following QC) |

3.2 | Detection of outlier loci

The neutral matrices calculated to generate "standardized allele frequencies" for BAYENV2 varied little within runs and when compared to the \( F_{ST} \) matrix calculated for the neutral loci; we therefore chose a single matrix for further calculations with BAYENV2. LFMM identified \( K = 2 \) as the most supported number of clusters (and thus latent factors) using GIF analysis, with values of 0.85 for AllEnv and 0.83 for CST. According to Frichot and François (2015), p-values in this analysis calibrate correctly when GIF approaches one.

Overall, LFMM identified more loci potentially under selection than BAYENV2. Combining the results from both programs, AllEnv identified 621 loci (8.7% of all loci) as under selection, whereas CST identified 285 loci (4.0% of all loci) as under selection; 250 loci were shared between the two datasets (Table 6). Using AllEnv, BAYENV2 detected 128 loci as putatively under selection (1.8% of total loci), whereas LFMM detected 511 (7.1% of total loci). Only 18 loci were common to both the BAYENV2 and LFMM sets. Using CST, BAYENV2 detected 72 loci (1.0% of total loci), whereas LFMM detected 218 (3.0% of total loci), with only five loci shared between the two methods. Within the BAYENV2 results, the AllEnv outlier list and CST outlier list shared 37 loci (Table S1). The LFMM analysis of AllEnv and CST overlapped completely in loci identified (Table S2). We also compared our combined outlier lists to the \( F_{ST} \)-based outliers reported in Van Wyngaarden et al. (2017) (112 outliers). The combined AllEnv outlier list and the \( F_{ST} \)-based list shared 53 loci, the combined CST outlier list and the \( F_{ST} \)-based list shared 35 loci, and all three lists shared 28 loci (Table S3).

3.3 | Patterns of genetic and environmental variation

PCA of all individuals and sets of outlier loci detected using AllEnv and CST both split north and south populations along the first PC, separating the populations into two clusters as seen in the BAYENV2 results (Figure 3). Using AllEnvOutlier, the first PC explained 2.38% of the total explainable variance in the model, and using CSTOutlier, the first PC explained 3.33% of the total explainable variance. The PCA on the population-specific allele frequencies for AllEnvOutlier and CSTOutlier

| TABLE 6 | (A) Matrix of the number of outlier loci detected in P. magellanicus out of 7163 total loci by the methods BAYENV2 and LFMM using two environmental datasets, AllEnv and CST. The number of loci shared between different environmental datasets and programs are italicized. (B) Combined total number of loci detected from two methods, BAYENV2 and LFMM, using two environmental datasets, AllEnv and CST. The number of loci shared between the different environmental datasets is italicized |
| Parameter | BAYENV2 | LFMM |
|---|---|---|---|---|
| (A) | AllEnv | CST | AllEnv | CST |
| BAYENV2 | 128 | 37 | 18 | – |
| CST | – | 72 | – | 5 |
| LFMM | – | – | 511 | 218 |
| (B) | AllEnv | CST | 621 | 250 |
| CST | – | 285 |
produced a similar clustering pattern; however, the first PC explained much more variance, with AllEnvOutlierPC1 explaining 26.47% of the total model variance and CSTOutlierPC1 explaining 31.93% of the total model variance.

The environmental data produced the same pattern of north–south population clustering for both datasets (AllEnv and CST, Figure 4). However, these PCAs further separated the southernmost population, Mid-Atlantic Bight (MDA), along the second PC. The first PC of the environmental data explained much more variance than in the genetic models, with AllEnvPC1 explaining 40.18% of the total model variance and CSTPC1 explaining 51.35%. Linear regressions between genetic and environmental data (i.e., AllEnvOutlierPC1 and AllEnvPC1 as well as CSTOutlierPC1 and CSTPC1) showed a strong and significant relationship (Figure 5), with adjusted $R^2$ values of .79 for AllEnv and .66 for CST, further indicating similar spatial patterns in genetic and environmental variation among our sample sites. The north–south population split can be seen in heat maps of standardized major allele frequency and standardized values for environmental variables in AllEnv and AllEnvOutlier and CST and CSTOutlier (Figure 6). In Figure 6b,d, lower standardized values can be seen in the four furthest north populations, and in Figure 6a,c, a similar split can be seen in standardized major allele frequencies, although only for a subset of alleles showing the strong clinal pattern driving the north–south split.

### 3.4 Environmental factors that influence genetic variation

To examine the effects of climate versus geography on the genetic variation within the outlier SNP loci, we selected five PCs from AllEnvPCA and four from CSTPCA for use as explanatory variables in RDA, each explaining more than 5% of the total variance in the PCA. In AllEnvPCA, the five selected axes explained 89.78% of the total model variance, and in CSTPCA, the four selected axes explained 88.96% of the total variance. Backwards stepwise variable selection on the RDA for AllEnv retained only AllEnvPC1 as an important explanatory variable, whereas selection on the RDA for CST retained both CSTPC1 and CSTPC4 (Figure 7). Both
models demonstrated significant relationships, despite low adjusted $R^2$ values (AllEnv, $R^2_{adj} = .15, p = .001$; CST, $R^2_{adj} = .23, p = .001$). Variance partitioning showed that climate explained a significant component of the model variation in both cases, explaining 32.36% of model variation in AllEnv (compared to 30.37% explained by geography and 37.28% explained as joint effects) and 41.34% of model variation in CST (compared to 21.27% explained by geography and 37.39% explained by joint effects).

The RDAs for AllEnv and CST both separated north and south population groups. AllEnv retained only one environmental PC axis, and we therefore show only one RDA axis in the plot (Figure 7a); however, this axis clearly divides the north and south populations. In CST, RDA1 divided north and south but further division among sample sites can be seen along RDA2, including separation of populations from Newfoundland and the Gulf of St. Lawrence (Figure 7b). Partial RDA, following conditioning of the genetic matrix on the distance between populations, no longer separated north and south populations once the effect of population separation distance was removed (Figure 8, AllEnv, $R^2_{adj} = .04, p = .06$; CST, $R^2_{adj} = .07, p = .03$). We expected this result given the strong relationship between environmental parameters and latitude in this region and the large latitudinal but small longitudinal span of the samples.

To choose environmental parameters to include in the multiple linear models, we examined variable weightings on the PC axes selected during RDA and retained the five most highly weighted variables from each axis. For all variables included in each global model, we calculated cumulative AICc weights and model-averaged parameter estimates (Table 7). Model selection using CSTEnv and all 10 selected environmental variables could not determine best fit models and provide accurate estimates for parameter weights and coefficients due to overfitting of the model. Upon further examination of the RDA results, CSTPC1 appeared more important in driving the north–south population split. We repeated our multiple linear regressions and model averaging using only the five most highly weighted variables from CSTEnvPC1. In all cases, model weights averaged over all possible iterations of the models containing a particular variable indicated surface average winter temperature as the most important variable. Surface minimum temperature (occurred in winter) and deep average winter temperature also ranked highly, suggesting that the coldest temperatures encountered by both juvenile and adult scallops may play an important structuring role for scallop populations. Parameter estimates for all three variables were positive; increased minimum temperatures in the model corresponded to larger values of the first PC (higher PC values match the south population cluster).

Using RF, we calculated the importance proportion for all environmental variables using both AllEnvOutlier and CST Outlier (Figure 9). Using AllEnvOutlier, deep average summer salinity, deep minimum salinity (occurred in spring), and deep maximum salinity (occurred in autumn) ranked as the most important environmental variables. Surface average autumn temperature, deep average winter temperature, and deep minimum temperature (occurred in winter) were also selected as important variables. CSTOutlier once again ranked salinity-associated variables as most important; however, deep average winter temperature and deep minimum temperature ranked highly and the importance proportions for CSTOutlier exceeded those from AllEnvOutlier.

### 3.5 Gene ontology

Blast2GO functionally annotated very few outlier loci. CSTOutlier determined annotation matches for only four loci (1.4% of loci), with a BLAST hit but no GO annotation at one further locus. In AllEnvOutlier, only five loci (0.8% of total loci) matched, with a BLAST hit but no GO annotation in one further locus. The two lists of outliers shared three matches, with GO annotations split between molecular function (calcium ion and carbohydrate binding) and metabolic processes (regulation of transcription and steroid hormone-mediated signaling). In CSTOutlier, GO annotation of the remaining locus identified a molecular function (oxidoreductase activity) and a metabolic process (oxidation–reduction process). In AllEnvOutlier, the GO annotations of the remaining two loci differed, one locus with molecular functions (oxidoreductase activity) and metabolic processes (oxidation–reduction process) and the other locus with several annotations (molecular function/catalytic activity, transferase activity, and folic acid binding, and metabolic processes/cellular metabolic processes) (Table 8).
4 | DISCUSSION

The identification of environmental factors regulating marine population structure can both inform fisheries management through the identification of management units and help predict species’ responses to environmental change. Here, we applied a landscape genomics approach using 7163 RAD-seq-derived SNPs (previously identified in Van Wyngaarden et al., 2017) and 90 environmental variables to identify oceanographic factors associated with a latitudinal genomic cline in sea scallops in eastern North America. Our results support the hypothesis that seasonal periods of extreme cold restrict gene flow and influence population structure in this species. This work builds on previous studies on population structure in *P. magellanicus* (Kennington et al., 2006; Owen & Rawson, 2013), particularly the identification of a major genomic discontinuity separating the north and south of the species range (Van Wyngaarden et al., 2017). Our multivariate analysis using the outlier loci and environmental variables identified minimum and average winter temperatures as the most important variables describing genetic variation among populations of the scallop, indicating that overwinter survival may strongly influence structure of these populations. We also identified minimum salinity as a potential structuring force, although to a lesser extent and affecting fewer populations than temperature changes over the range of the species. Overall, the observed genomic and environmental correlations support the hypothesis of latitudinal structuring driven predominantly by ocean temperature.

4.1 | Environmental variables driving adaptation

Our results highlight ocean temperature as a critical environmental factor contributing to population structuring of the sea scallop. The sea scallop’s distribution spans almost 10° latitude encompassing an...
extremely large range of environmental conditions (approximately 5-10°C difference in temperatures year-round), primarily caused by prevailing currents (Townsend et al., 2006). The Labrador Current, a cold Arctic current, flows south from the coasts of northern Canada and Greenland, splitting around Newfoundland and circulating through the Gulf of St. Lawrence (Townsend et al., 2006). In contrast, the warm Gulf Stream moves north from the Gulf of Mexico along the east coast of North America. These two currents meet and move roughly offshore around Nova Scotia, exposing scallop populations to large differences in water temperature (and other oceanographic variables) in different areas of their range (Townsend et al., 2006). Our environmental PCAs clearly detected the differences in environment associated with these currents. The first PC in our environmental PCAs illustrates the split between northern and southern populations and for both AllEnv and CST explains more than 40% of the variation in the environmental data.

Using \( F_{ST} \)-based outlier detection methods, Van Wyngaarden et al. (2017) identified a strong genetic population separation related to these currents and the other oceanographic and environmental features in the region; populations in the north, generally exposed to colder temperatures, clustered separately from the southern populations, which are often exposed to warmer temperatures. This genetic split also clearly appears in the first PC of our genetic outlier PCAs, however the first PC in both of our analyses explains little variance, especially when compared with outliers used in Van Wyngaarden et al. (2017). This difference in explained variance among the genetic PCs likely results from the method used to detect outlier loci given some differences between the outlier lists detected here and in Van Wyngaarden et al. (2017) (Table S3). Van Wyngaarden et al. (2017) used BayeScan (Foll & Gaggiotti, 2008), which uses an \( F_{ST} \)-based method to detect outliers and generally selects the most divergent loci. In comparison, both BAYENV2 and LFMM use environmental correlations to detect outlier loci. Although these methods may also identify highly divergent loci as outliers, if a highly divergent locus (likely to be detected by BayeScan) does not correlate with the environmental variation captured in our environmental dataset, it would not be included in the final outlier list and would not contribute to the variance explained by the first PC in our genetic PCAs.

**FIGURE 7** Redundancy analysis plots for loci detected as potentially under selection through environmental correlation with (a) AllEnv (90 environmental variables, \( n = 621 \) loci), (b) CST (36 environmental variables, \( n = 285 \) loci) in 12 populations of *Placopecten magellanicus*. Explanatory variables (arrows) were principal components axes from PCA on (a) AllEnv and (b) CST, retained as important following backwards stepwise variable selection.

**FIGURE 8** Partial redundancy analysis plots for loci detected as potentially under selection through environmental correlation with (a) AllEnv (90 environmental variables, \( n = 621 \) loci), (b) CST (36 environmental variables, \( n = 285 \) loci) in 12 populations of *Placopecten magellanicus*. Explanatory variables used were principal components axes from PCA on (a) AllEnv and (b) CST, retained following backwards stepwise variable selection. The genetic matrix was conditioned on the distance between populations to reduce the effects of geographic separation between populations.
Van Wyngaarden et al. (2017) documented a genetic discontinuity between northern and southern scallop populations in all SNP loci, although the magnitude of differentiation was significantly higher at outlier loci. This pattern indicates that although neutral processes may play a role in population structuring, selection plays a dominant structuring role across the geographic range of the sea scallop. Considering this finding, we focused on environmental-based outlier detection methods to identify putative causes of population structure in the sea scallop. Of the environmental variables we examined, temperature primarily drives the separation of northern and southern populations and the coldest temperatures (winter and minimum) differ most between these groups. This finding is consistent with other studies in the North Atlantic, where temperature variations (particularly with latitude) represent some of the strongest differences among regions (Townsend et al., 2006); temperature is likely the dominant selective force in this region and among scallop populations. Strong correlations between genetic variation and ocean temperatures have been observed in many North Atlantic fish species (e.g., Berg et al., 2015; Bourret, Dionne, Kent, Lien, & Bernatchez, 2013; Bradbury et al., 2010, 2014; Limborg et al., 2012) and other North Atlantic invertebrates, specifically in Benestan et al. (2016) where the population structure of American lobster (Homarus americanus) was also found to be driven by minimum annual water temperatures.

**TABLE 7** Cumulative Akaike information criterion model weights (\(\Sigma \omega_i\)) and model-averaged parameter estimates (full: variables assumed to be present in all models with a coefficient of 0 in some cases; subset: variables only present in models where the coefficient was not 0) in models predicting whether genetic variation in outlier loci among populations of *Placopecten magellanicus* is a function of environmental variation. (A) Outlier loci were detected through correlations with an environmental dataset of 90 variables (AllEnv, \(n = 621\) loci). Environmental variables were selected following the results of principal components analysis and redundancy analysis. (B) Outlier loci were detected through correlations with an environmental dataset of 36 variables (CST, \(n = 285\) loci). Environmental variables were selected following the results of principal components analysis and redundancy analysis. (C) Outlier loci were the same used in (A). Environmental variables were selected following the results from (A) and (B). (D) Outlier loci were the same used in (C). Environmental variables were selected following the results from (A) and (B).

| Parameter                  | \(\Sigma \omega_i\) | Model-averaged parameter estimates | Full | Subset       |
|----------------------------|----------------------|-----------------------------------|------|--------------|
| (A) SurfAvWinTemp          | 0.426                | 0.6767452                         | 1.5889694 |
| SurfMinTemp                | 0.315                | 0.1673587                         | 0.5337035 |
| SurfAvAutSal               | 0.272                | 0.2465678                         | 0.9065121 |
| DepMinSiO\(_3\)            | 0.256                | 0.2009489                         | 0.7841225 |
| DepAvAutSal                | 0.152                | 0.02467925                        | 0.1628929 |
| SurfAvWinTemp              | 0.437                | 0.5586436                         | 1.2785748 |
| SurfAvSprTemp              | 0.434                | 0.5879726                         | 1.6505098 |
| SurfAvSumTemp              | 0.343                | -0.4611794                        | -1.3452456 |
| DepMinTemp                 | 0.275                | 0.3441115                         | 1.2523863 |
| DepAvAutSal                | 0.109                | -0.001612218                      | -0.014871888 |
| (C) SurfAvWinTemp          | 0.643                | 1.031135                          | 1.604291   |
| SurfAvWinTemp              | 0.282                | 0.0493947                         | 0.1747112   |
| SurfAvSprTemp              | 0.142                | 0.1584858                         | 1.0963318   |
| SurfAvSumTemp              | 0.141                | -0.09856687                       | -0.69285742 |
| DepAvSprTemp               | 0.103                | 0.04809931                        | 0.45494143  |
| SurfAvTemp                 | 0.095                | 0.0477454                        | 0.46824153  |
| SurfAvSumTemp              | 0.083                | 0.01956854                        | 0.23171602  |
| SurfAvSprTemp              | 0.080                | -0.01290984                       | -0.1627252   |
| (D) SurfAvWinTemp          | 0.587                | 0.6509583                         | 1.10358     |
| SurfAvWinTemp              | 0.302                | 0.1932762                         | 0.6457666   |
| SurfAvSprTemp              | 0.131                | 0.004951037                       | 0.067160279 |
| SurfAvSumTemp              | 0.121                | 0.08023971                        | 0.5923327   |
| SurfAvTemp                 | 0.095                | 0.01497057                        | 0.16543456  |
| SurfAvSprTemp              | 0.092                | -0.02916119                      | -0.30036982 |
| SurfAvSumTemp              | 0.089                | 0.02933703                       | 0.312122   |
| SurfAvSprTemp              | 0.072                | 0.07923188                        | 0.63938784   |

**FIGURE 9** Proportion of importance (average per variable importance/importance sum of all variables) for the top 15 environmental variables determined using random forest and (a) AllEnv and AllEnvOutlier, and (b) CST and CSTOutlier.
However, in many regions other environmental features often co-vary with temperature (e.g., salinity or ChlA) and in some analyses temperature may act as an unintentional proxy for the true selective force (a species may appear to adapt to temperature when in fact they are experiencing selection due to another variable such as ocean productivity). This may have particular relevance for the sea scallop, because our sampling locations and temperature gradient both span the same north–south axis. There is also some evidence of a slight north–south salinity gradient, with the lowest salinities in MGD and NTS and the highest salinity in MDA (Figure 6b,c). Although clear associations between genetic variation and temperature have been reported in several other species, including Pacific invertebrates (Pespeni & Palumbi, 2013), studies also demonstrate genomic adaptation to environmental gradients other than temperature, such as adaptation to salinity gradients in several Baltic Sea species (Berg et al., 2015; Limborg et al., 2012; Sjöqvist et al., 2015). In our analyses, in addition to cold temperatures RF analysis also identified salinity as an important environmental variable, likely driven by very low salinity values at NTS and MGD in the Gulf of St. Lawrence. Our RF analyses used allele frequencies across all populations; however, by handling covariation between environmental data RF may have been able to detect the smaller-scale variation associated with salinity in the Gulf of St. Lawrence that may have been masked by the strong temperature associations influencing the multiple linear regression analysis. Overall, genetic variation reflects the geographic patterns present in the significant environmental variables (Figure 10). When plotted against pairwise $F_{ST}$ (calculated using ARLEQUIN v.3.5 (Excoffier & Lischer, 2010)), the winter and minimum water temperatures clearly differ between Van Wyngaarden et al. (2017)’s northern and southern population groups. Additionally, NTS has a higher differentiation from SUN than other nearby populations, potentially reflecting a response to the lower minimum and summer salinities present at that location. Further sampling along a salinity gradient not confounded by a temperature gradient may help to disentangle the covarying effects.

### 4.2 | Mechanisms of adaptation

The genomic associations with ocean temperature during periods of extreme cold (i.e., winter) suggest temperature-associated mortality may significantly structure sea scallop populations. Sea scallops reproduce via broadcast spawning, generally in the autumn, although timing varies along their range. Given that scallops tend to spawn in the warmest water (Thompson, 1977), generally between August and October (Beninger, 1987; Langton, Robinson, & Schick, 1987; Naidu, 1970), and they likely settle before December (Naidu & Robert, 2006), a link between winter temperatures and larval mortality appears unlikely. Our analyses point to the overwinter survival of juvenile scallops as a potentially important structuring force limiting the effective dispersal of scallops between our northern and southern population groups, rather than selective mortality of planktonic larval scallops, and future experimental studies on larval and juvenile scallops may help to clarify this possibility. Some evidence suggests that temperatures experienced by adults can help ensure a healthy larval year class (Dickie, 1955; DuPaul, Kirkley, & Schmitzer, 1989; Kirkley & Dupaul, 1991; Langton et al., 1987; Macdonald & Thompson, 1985). Interestingly, our study identified surface temperature rather than temperature at depth as the most important driver of selection, contrary to expectations of juvenile scallop survival. One possible explanation is that deep temperature values are often estimated or provided as a range at collection sites, presumably reducing accuracy of those measurements relative to those for surface temperature. Our Blast2GO results identified possible genetic matches with several cellular processes, which may be temperature dependent, highlighting potential mechanisms of thermal adaptation in the sea scallop. Unfortunately, the lack of

| Environmental data | Locus name | GO name |
|--------------------|------------|---------|
| AllEnv             | 16087_68   | F: catalytic activity; P: metabolic process; F: transferase activity; F: folic acid binding; P: cellular metabolic processes |
| AllEnv             | 24384_24   | F: oxidoreductase activity; P: metabolic process; P: oxidation–reduction process |
| AllEnv and CST     | 12228_13   | P: regulation of transcription; P: steroid hormone-mediated signaling pathway |
| AllEnv and CST     | 20561_41   | F: carbohydrate binding |
| AllEnv and CST     | 25748_78   | F: calcium ion binding |
| CST                | 15446_21   | F: oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced pteridine as one donor, and incorporation of one atom of oxygen; P: oxidation–reduction process |

**Table 8** Blast2GO functional annotation of outlier SNP loci from 12 populations of *Placopecten magellanicus*. Outliers were detected through environmental correlations with 90 environmental variables (AllEnv, 621 loci) or a subset of 26 environmental variables (CST, 285 loci)
available genetic resources (i.e., reference genome) for the sea scallop impedes our ability to evaluate fully the functional importance of the loci identified here. Until improved genetic data resources for the sea scallop or related species are available, any conclusions drawn from annotation results are preliminary.

### 4.3 |Alternative contributions to population structure

Despite the clear association observed with ocean temperature and population structure, selective processes may not be the sole driving mechanism of population structure in the sea scallop. Neutral oceanographic barriers to connectivity have been documented in other marine bivalves in the Northwest Atlantic, including *Mytilus* sp. in the Gulf of Maine (Yund et al., 2015). As described in a review by Bierne, Welch, Loire, Bonhomme, and David (2011), in many cases local adaptation alone may not explain the genetic structure detected among populations or the geographic location of the strong break between population clusters. Tension zones (caused by endogenous barriers to gene flow) may have arisen independent of selection caused by environmental variation along the range of the species, potentially influencing the separation of population groups between the north and south of the species range. These tension zones may associate with environmental clines, and a combination of both endogenous and exogenous barriers (tension zones and selection) could contribute to the detected structure. This scenario could also reinforce local adaptation associated with environmental adaptation (Sexton, Hangartner, & Hoffmann, 2014; Shafer & Wolf, 2013), furthering differentiation between regions. The detection of a genetic discontinuity between northern and southern populations of the sea scallop in both outlier and neutral loci (Van Wyngaarden et al., 2017) suggests that a combination of neutral and selective forces leads to the population structure detected, although the magnitude of the genetic break in outlier loci is larger than that of neutral loci indicating that selective forces play a larger structuring role than neutral forces in the sea scallop.

The sea scallop range encompasses an area of complex oceanography and several processes could contribute to the neutral genetic separation of populations including potential current-related fronts that may prevent larval movement between regions, upwelling and water movement related to the continental shelf, and storm mixing along the coast (Townsend et al., 2006). These factors may inhibit the successful movement, settlement, and growth of larvae and can be difficult to accurately incorporate into models of connectivity that try to calculate larval dispersal. Additionally, larval behavior significantly impacts dispersal in many cases (Shanks, 2009) and can be difficult to accurately model and evaluate. Using postsettlement genetic structure to determine what processes influence connectivity among sea scallop populations, our methods inherently account for the effects of larval behavior and complexity when drawing conclusions. Although we believe our results to be robust to complications of neutral population structure and geographic distance, additional sampling (especially from populations at the same latitude) will help to more thoroughly separate the joint effects of climate and geography on scallop population structure as it may allow sampling of populations with a similar climatic profile at varying separation distances.

### 4.4 |Challenges and limitations

Many reviews on environmental association studies recommend removing the effects of neutral population structure to fully assess the effect of selection on population structure in natural systems (e.g., Rellstab, Gugerli, Eckert, Hancock, & Holderegger, 2015) and accounting for geographic distance and isolation by distance when examining potential isolation by ecology (e.g., Shafer & Wolf, 2013); however, this is a particular challenge in our system. Because a single north–south population split characterizes our sample sites rather than a classic isolation-by-distance pattern (Van Wyngaarden et al., 2017), geographic distance among populations may not influence our results the way it would in a system characterized by a classic stepping-stone pattern. Our samples also align along the north–south axis of the population range providing few opportunities to examine the effects of distance between samples without also removing the effects of latitude. To minimize the potential bias of neutral population structure on our results, we focused our analysis solely on outlier loci potentially under selection in the genome, likely making our analyses less prone to the confounding effects of neutral population structure. We also compared the results of RDA and pRDA, which controls for geographic distance among populations. Even when controlling for geographic distance, our results nonetheless indicate climate as a significant population structuring force, although the patterns of population clustering change slightly.

Another source of bias in population genomics studies is the effect of age-related structuring in population samples, which has been documented in scallops previously (Owen & Rawson, 2013), potentially due to recruitment events or yearly environmental fluctuations. Although our samples were collected over a relatively short time period (2011–2013), we made attempts to cover multiple age classes to avoid this issue.

Our analyses pinpointed potential environmental influences on sea scallop population structure; however, annotating the outlier SNPs of interest remains challenging. Although RAD-seq generates vast quantities of SNPs in organisms without reference genomes (Benestan et al., 2015; Catchen et al., 2013; Hohenlohe et al., 2012; Reitzel, Herrera, Layden, Martindale, & Shank, 2013), the lack of more detailed genetic resources makes inference on the causal mechanisms contributing to local adaptations in sea scallops difficult, as noted by our lack of GO matches. Fortunately, with continued development of resources for *P. magellanicus* and related species, future studies will likely identify and study the features most important in characterizing sea scallop population structure.

### 5 |CONCLUSIONS

Our results show that ocean climate plays a role in structuring populations of sea scallops, particularly the influence of the coldest
temperatures experienced. The association with coldest temperatures points to the overwinter survival of juvenile scallops as a structuring force rather than survival of larval scallops, contrary to what might be expected for broadcast spawning marine species. This work and similar landscape (or seascape) genetic studies highlight the possibility that local adaptation and the differential survival of dispersers (rather than solely limited dispersal) may have greater impact on the population structure of marine species than previously hypothesized. Our results can be useful in the effective management of *P. magellanicus* by helping managers in both Canada and the United States accurately determine geographic sources of larvae for exploited populations and predict the potential reactions of this species to a changing ocean climate, particularly with changes to the location and strength of dominant currents. Our results also provide an important starting point for future studies. If temperature drives variation in the reproductive rates of scallops, then increasing water temperatures associated with global warming may alter scallop reproductive cycles and subsequent recruitment (Robinson, Martin, Chandler, & Parsons, 2007). Genetic and genomic studies to examine further effects of selection on population structure in scallops, in tandem with experimental studies to identify adaptations among scallop populations, may be critical for predicting how the species will react to future climate change and harvesting pressures. Additionally, access to further genetic resources will continue to improve identification of the genes and pathways involved in adaptation and population structuring among sea scallop populations.

**ACKNOWLEDGMENTS**

The authors thank staff at Fisheries and Oceans Canada, the Maine Department of Marine Resources, and NOAA, as well as private scallop harvesters for their assistance with sample collection. They also thank the Aquatic Biotechnology Lab at the Bedford Institute of Oceanography for all genetic sample processing and sequencing preparation. Drs. Catherine Johnson, Peter Galbraith, and Pierre Pepin as well as Andrew Cogswell and Roger Pettipas (DFO) and Dr. Jon Hare (NOAA) were extremely helpful in providing the oceanographic data used in this study. Dr. Ryan Stanley provided excellent guidance and scripts for making heat maps in R. Funding was provided by NSERC Discovery Grants to Drs. Ian Bradbury and Paul Snelgrove, Genomic Resource Development Initiative grants (Phase V and VI) to Drs. Ian Bradbury and Claudio DiBacco, an NSERC Strategic Project Grant on fisheries genomics, and the School of Graduate Studies at Memorial University (funding to Mallory Van Wyngaarden).
DATA ACCESSIBILITY

All raw sequences are available at NCBI SRA Bioproject number PRJNA340326, Biosample numbers SAMN05712457–SAMN05712468. The full outlier lists are available in Tables S1 and S2. The sample site distance matrices, final environmental metrics, the 7,216 SNPs selected before HWE filtering (VCF format), sequence sample metadata, and annotated R scripts of the main statistical analyses are available on Dryad at http://datadryad.org/resource/doi:10.5061/dryad.c15v5.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

IB, PS, and CDB planned and coordinated scallop sample collection. LH completed the sample preparation and RAD-seq library preparation. NRE processed the raw RAD-seq data and prepared the final genotype tables. MVW completed the analysis and wrote the manuscript. MVW, IB, PS, and CDB planned the analyses. MVW, IB, PS, and CDB read, edited, and approved the manuscript. LZ completed the random-forest analysis with guidance and advice from RGB. All authors submitted comments, edits, and suggestions during the final preparation of the manuscript.

ORCID

Malory Van Wyngaarden http://orcid.org/0000-0002-3789-2351

REFERENCES

Allendorf, F. W., Hohenlohe, P. A., & Luikart, G. (2010). Genomics and the future of conservation genetics. Nature Reviews Genetics, 11, 697–709. https://doi.org/10.1038/nrg2844
Arnold, T. W. (2010). Uninformative parameters and model selection using Akaike's Information Criterion. The Journal of Wildlife Management, 74, 1175–1178. https://doi.org/10.1002/jwmg.21236
Barton, K. (2014). MuMIn: multi-model inference. Retrieved from https://cran.r-project.org/web/packages/MuMIn/index.html.
Benestan, L., Gosselin, T., Perrier, C., Sainte-Marie, B., Rochette, R., & Bernatchez, L. (2015). RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American lobster (Homarus americanus). Molecular Ecology, 24, 3299–3315. https://doi.org/10.1111/mec.13245
Benestan, L., Quinn, B. K., Maaroufi, H., Laporte, M., Clark, F. K., Greenwood, S. J., ... Bernatchez, L. (2016). Seascape genomics provides evidence for thermal adaptation and current-mediated population structure in American lobster (Homarus americanus). Molecular Ecology, 25, 5073–5092. https://doi.org/10.1111/mec.13811
Beninger, P. G. (1987). A qualitative and quantitative study of the reproductive cycle of the giant scallop, Placopecten magellanicus, in the Bay of Fundy (New Brunswick, Canada). Canadian Journal of Zoology, 65, 495–498. https://doi.org/10.1139/z87-077
Berg, P. R., Jentoft, S., Star, B., Ring, K. H., Knutsen, H., Lien, S., ... André, C. (2015). Adaptation to low salinity promotes genomic divergence in Atlantic cod (Gadus morhua L.). Genome Biology and Evolution, 7, 1644–1663. https://doi.org/10.1093/gbe/evv093
Bierne, N., Welch, J., Loire, E., Bonhomme, F., & David, P. (2011). The coupling hypothesis: Why genome scans may fail to map local adaptation genes. Molecular Ecology, 20, 2044–2072. https://doi.org/10.1111/j.1365-294X.2011.05080.x
Bourret, V., Dionne, M., Kent, M. P., Lien, S., & Bernatchez, L. (2013). Landscape genomics in Atlantic salmon (Salmo salar): Searching for gene-environment interactions driving local adaptation. Evolution, 67, 3469–3487.
Bradbury, I. R., Hamilton, L. C., Robertson, M. J., Bourgeois, C. E., Mansour, A., & Dempson, J. B. (2014). Landscape structure and climatic variation determine Atlantic salmon genetic connectivity in the Northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences, 71, 246–258. https://doi.org/10.1139/cjfas-2013-0240
Bradbury, I. R., Hubert, S., Higgins, B., Borza, T., Bowman, S., Paterson, I. G., ... Hutchings, J. A. (2010). Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in response to temperature. Proceedings of the Royal Society B-Biological Sciences, 277, 3725–3734. https://doi.org/10.1098/rspb.2010.0985
Bradbury, I. R., Hubert, S., Higgins, B., Bowman, S., Borza, T., Paterson, I. G., ... Hutchings, J. A. (2013). Genomic islands of divergence and their consequences for the resolution of spatial structure in an exploited marine fish. Evolutionary Applications, 6, 450–461. https://doi.org/10.1111/eaa.12026
Breiman, L. (2001). Random forests. Machine Learning, 45, 5–32. https://doi.org/10.1023/A:1010933404324
Brieuc, M. S. O., Ono, K., Drinan, D. P., & Naish, K. A. (2015). Integration of random forest with population-based outlier analyses provides insight on the genomic basis and evolution of run timing in Chinook salmon (Oncorhynchus tshawytscha). Molecular Ecology, 24, 2729–2746. https://doi.org/10.1111/mec.13211
Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. H. (2011). Stacks: Building and genotyping loci de novo from short-read sequences. G3-Genes, Genomes, Genetics, 1, 171–182.
Catchen, J., Bassham, S., Wilson, T., Currey, M., O’Brien, C., Yeates, Q., & Cresko, W. A. (2013). The population structure and recent colonization history of Oregon threespine stickleback determined using restriction-site associated DNA-sequencing. Molecular Ecology, 22, 2864–2883. https://doi.org/10.1111/mec.12330
Clarke, L. M., Munch, S. B., Thorrold, S. R., & Conover, D. O. (2010). High connectivity among locally adapted populations of a marine fish (Menidia menidia). Ecology, 91, 3526–3537. https://doi.org/10.1890/09-0548.1
Conesa, A., Gótz, S., García-Gómez, J. M., Terol, J., Talón, M., & Robles, M. (2005). Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics, 21, 3674–3676. https://doi.org/10.1093/bioinformatics/bti160
Conover, D. O., Clarke, L. M., Munch, S. B., & Wagner, G. N. (2006). Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. Journal of Fish Biology, 69, 21–47. https://doi.org/10.1111/j.1095-8649.2006.01274.x
Coop, G., Witonsky, D., Di Rienzo, A., & Pritchard, J. K. (2010). Using environmental correlations to identify loci underlying local adaptation. Genetics, 185, 1411–1423. https://doi.org/10.1534/genetics.110.114819
Corander, J., Majander, K. K., Cheng, L., & Merila, J. (2013). High degree of cryptic population differentiation in the Baltic Sea herring Clupea harengus. Molecular Ecology, 22, 2931–2940. https://doi.org/10.1111/mec.12174
Davies, K. T. A., Gentleman, W. C., DiBacco, C., & Johnson, C. L. (2014). Semi-annual spawning in marine scallops strengthens larval recruitment and connectivity on Georges Bank: A model study. Marine Ecology Progress Series, 516, 209–227. https://doi.org/10.3354/meps10975
De Wit, P., & Palumbi, S. R. (2013). Transcriptome-wide polymorphisms of red abalone (Haliotis rufescens) reveal patterns of gene flow and
Dickie, L. M. (1955). Fluctuations in abundance of the giant scallop, Placopecten magellanicus (Gmelin), in the Digby area of the Bay of Fundy. *Journal of the Fisheries Research Board of Canada*, 12, 797–856. https://doi.org/10.1139/f55-045

DuPaul, W. D., Kirkley, J. E., & Schmitzter, A. C. (1989). Evidence of a semi-annual reproductive cycle for the sea scallop, Placopecten magellanicus (Gmelin 1791) in the Mid-Atlantic region. *Journal of Shellfish Research*, 8, 173–178.

Dyer, R. J. (2014). An introduction to the gstudio package. Retrieved from http://dyerlab.github.io/gstudio/.

Etter, P. D., Bassham, S., Hohenlohe, P. A., Johnson, E. A., & Cresko, W. A. (2011). SNP discovery and genotyping for evolutionary genetics using RAD sequencing. In V. Origogozo, & M. V. Rockman (Eds.), *Molecular methods for evolutionary genetics* (pp. 157–178). New York, NY: Springer.

Etter, P. D., Preston, J. L., Bassham, S., Cresko, W. A., & Johnson, E. A. (2011). Local de novo assembly of RAD paired-end contigs using short sequencing reads. *PloS ONE*, 6, 10.

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Faircloth, B. C., & Glenn, T. C. (2012). Not all sequence tags are created equal: Designing and validating sequence identification tags robust to indels. *PloS ONE*, 7, 11.

Foll, M., & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics*, 180, 977–993. https://doi.org/10.1534/genetics.108.092221

Frichot, E., & François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods in Ecology and Evolution*, 8, 925–929. https://doi.org/10.1111/2041-210X.12382

Frichot, E., Schoville, S. D., Bouchard, G., & François, O. (2013). Testing for associations between loci and environmental gradients using latent factor mixed models. *Molecular Biology and Evolution*, 30, 1687–1699. https://doi.org/10.1093/molbev/msm063

Frichot, E., Schoville, S. D., de Villemereuil, P., Gaggiotti, O. E., & François, O. (2015). Detecting adaptive evolution based on association with ecological gradients: Orientation matters!. *Heredity*, 115, 22–28. https://doi.org/10.1038/hdy.2015.7

Gagnaire, P.-A., Broquet, T., Aurelle, C., Viard, F., Souissi, A., Bonhomme, F., ... Bierne, N. (2015). Using neutral, selected and hitchhiker loci to assess connectivity of marine populations in the genomic era. *Evolutionary Applications*, 8, 769–786. https://doi.org/10.1111/eva.12288

Gaithier, M. R., Bernal, M. A., Coleman, R. R., Bowen, B. W., Jones, S. A., Simion, W. B., & Rocha, L. A. (2015). Genomic signatures of geographic isolation and natural selection in coral reef fishes. *Molecular Ecology*, 24, 1543–1557. https://doi.org/10.1111/mec.13129

Google (2013). Google earth. Version 7.1.2.2041. Menlo Park, CA: Google Inc.

Gregory, D. N. (2004). Climate: A database of temperature and salinity observations for the Northwest Atlantic. Fisheries and Oceans Canada.

Guenter, T., & Coop, G. (2013). Robust identification of local adaptation from allele frequencies. *Genetics*, 195, 205. https://doi.org/10.1534/genetics.113.152462

Hancock, A. M., Witonsky, D. B., Ehler, E., Alkorta-Aranburu, G., Beall, C., Gembremedhin, A., ... Di Rienzo, A. (2010). Human adaptations to diet, subsistence, and ecoregion are due to subtle shifts in allele frequency. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 8924–8930. https://doi.org/10.1073/pnas.0914665107

Hauser, L., & Carvalho, G. R. (2008). Paradigm shifts in marine fisheries genetics: Ugly hypotheses stain by beautiful facts. *Fish and Fisheries*, 9, 333–362. https://doi.org/10.1111/j.1467-2979.2008.00299.x

Hecht, B. C., Matala, A. P., Hess, J. E., & Narum, S. R. (2015). Environmental adaptation in Chinook salmon (Oncorhynchus tshawytscha) throughout their North American range. *Molecular Ecology*, 24, 5573–5595. https://doi.org/10.1111/mec.13409

Hellberg, M. E. (2009). Gene flow and isolation among populations of marine animals. *Annual Review of Ecology Evolution and Systematics*, 40, 291–310. https://doi.org/10.1146/annurev.ecolsys.110308.120223

Jombart, T. (2008), adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Kenchington, E. L., Patwary, M. U., Zourovs, E., & Bird, C. J. (2006). Genetic differentiation in relation to marine landscape in a broadcast-spawning bivalve mollusc (*Placopecten magellanicus*). *Molecular Ecology*, 15, 1781–1796. https://doi.org/10.1111/j.1365-294X.2006.02915.x

Kirkley, J. E., & Dupaul, W. D. (1991). Temporal variation in spawning behavior of sea scallops, *Placopecten magellanicus* (Gmelin 1791) in the Mid-Atlantic resource area. *Journal of Shellfish Research*, 10, 389–398.

Lamichhaney, S., Barrio, A. M., Rafati, N., Sundström, G., Rubin, C. J., Gilbert, E. R., ... Grabherr, M. (2012). Population-scale sequencing reveals genetic differentiation due to local adaptation in Atlantic herring. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 19345–19350. https://doi.org/10.1073/pnas.1216128109

Langton, R. W., Robinson, W. E., & Schick, D. (1987). Fecundity and reproductive output of sea scallops *Placopecten magellanicus* from the Gulf of Maine. *Marine Ecology Progress Series*, 37, 19–25. https://doi.org/10.3354/meps03702

Liaw, A., & Wiener, M. (2002). Classification and regression by randomForest. *R News*, 2, 18–22.

Limborg, M. T., Helyar, S. J., De Bruyn, M., Taylor, M. I., Nielsen, E. E., Ogden, R. O. B., ... Bekkevold, D. (2012). Environmental selection on transcriptome-derived SNPs in a high gene flow marine fish, the Atlantic herring (*Clupea harengus*). *Molecular Ecology*, 21, 3686–3703. https://doi.org/10.1111/j.1365-294X.2012.05639.x

Macdonald, B. A., & Thompson, R. J. (1985). Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus*. II. Reproductive output and total production. *Marine Ecology Progress Series*, 25, 295–304. https://doi.org/10.3354/meps02529

Milano, I., Babbucci, M., Cariani, A., Atanassova, M., Bekkevold, D., Carvalho, G. R., ... Hansen, J. (2014). Outlier SNP markers reveal fine-scale genetic structuring across European hake populations (*Mullusculus merloussis*). *Molecular Ecology*, 23, 118–135. https://doi.org/10.1111/mec.12568

Moura, A. E., Kenny, J. G., Chaudhuri, R., Hughes, M. A., Welch, J., Reisinger, R. R., ... Hoelzel, A. R. (2014). Population genomics of the killer whale indicates ecotype evolution in sympathy involving both selection and drift. *Molecular Ecology*, 23, 5179–5192. https://doi.org/10.1111/mec.12929

Naidu, K. S. (1970). Reproduction and breeding cycle of giant scallop *Placopecten magellanicus* (Gmelin) in the Port-Au-Port Bay, Newfoundland. *Canadian Journal of Zoology*, 48, 1003–1012. https://doi.org/10.1139/z70-176

Naidu, K. S., & Robert, G. (2006). Fisheries Sea Scallop, *Placopecten magellanicus*. In S. Shumway, & G. J. Parsons (Eds.), *Scallop: Biology, ecology and aquaculture* (pp. 869–905). Amsterdam, The Netherlands: Elsevier. https://doi.org/10.1016/s0167-9309(06)80042-6
SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Van Wyngaarden M, Snelgrove PVR, DiBacco C, et al. Oceanographic variation influences spatial genomic structure in the sea scallop, Placopecten magellanicus. Ecol Evol. 2018;8:2824–2841. https://doi.org/10.1002/ece3.3846