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Seascape genomics reveals adaptive divergence in a connected and commercially important mollusc, the greenlip abalone (*Haliotis laevigata*), along a longitudinal environmental gradient

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Abstract

Populations of broadcast spawning marine organisms often have large sizes and are exposed to reduced genetic drift. Under such scenarios, strong selection associated with spatial environmental heterogeneity is expected to drive localized adaptive divergence, even in the face of connectivity. We tested this hypothesis using a seascape genomics approach in the commercially important greenlip abalone (*Haliotis laevigata*). We assessed how its population structure has been influenced by environmental heterogeneity along a zonal coastal boundary in southern Australia linked by strong oceanographic connectivity. Our data sets include 9,109 filtered SNPs for 371 abalones from 13 localities and environmental mapping across ~800 km. Genotype–environment association analyses and outlier tests defined 8,786 putatively neutral and 323 candidate adaptive loci. From a neutral perspective, the species is better represented by a metapopulation with very low differentiation (global F_{ST} = 0.0081) and weak isolation by distance following a stepping-stone model. For the candidate adaptive loci, however, model-based and model-free approaches indicated five divergent population clusters. After controlling for spatial distance, the distribution of putatively adaptive variation was strongly correlated to selection linked to minimum sea surface temperature and oxygen concentration. Around 80 candidates were annotated to genes with functions related to high temperature and/or low oxygen tolerance, including genes that influence the resilience of abalone species found in other biogeographic regions. Our study includes a documented example about the uptake of genomic information in fisheries management and supports the hypothesis of adaptive divergence due to coastal environmental heterogeneity in a connected metapopulation of a broadcast spawner.

KEYWORDS

climate change, ddRAD-seq, ecological genomics, landscape genomics, marine protected areas (MPAs), population connectivity, southern Australia

1 | INTRODUCTION

Environmental conditions impact on the genetic architectures and evolutionary trajectories of metapopulations by imposing selective pressures and influencing migration. Widely distributed species are often found across heterogeneous environments, which can result in patterns of localized adaptive divergence of populations (Nosil, 2012; Savolainen, Lascoux, & Merilä, 2013). Although such adaptive clusters may originate at relatively small spatial and temporal scales, they are important in evolutionary and ecological contexts, as well as for biodiversity management (Allendorf, Luikart, & Aitken, 2012; Bernatchez et al., 2017; Nielsen, Hemmer-Hansn, Larsen, & Bekkevold, 2009; Russello, Kirk, Frazer, & Askey, 2012). For harvested aquatic species, understanding how organisms adapt and respond to the environment can inform on population monitoring, stock boundaries, restocking and stock enhancement programmes, and fisheries induced evolution (Gonçalves da Silva, Appleyard, & Upston, 2015; Besnier et al., 2015; Pavey et al., 2015; reviewed in Bernatchez et al., 2017). As many adaptive traits in the wild are also important production traits (e.g., growth rate, disease resistance, tolerance to extreme events), there are also potential benefits for improving aquaculture and biosecurity practices (Bernatchez et al., 2017; Gjedrem, 2012). Knowledge about adaptive divergence is also fundamentally important for understanding the molecular basis of climate-induced micro-evolution, allowing predictions about the distribution and abundance of species under future climate scenarios (Attard et al., 2018; Brauer, Hammer, & Beheregaray, 2016; Gonzalez, Ronce, Ferriere, & Hochberg, 2013; Hansen, Olivieri, Waller, & Nielsen, 2012).

The identification of genetic subdivisions and adaptively divergent units is particularly challenging in marine organisms (Allendorf et al., 2012; Russello et al., 2012). Many marine species have large population sizes and considerable dispersal potential, which often result in high genetic diversity and weak genetic differentiation (Ovenden, 2013). This is particularly true for broadcast spawning organisms, for which it is difficult to determine whether weak genetic differentiation is due to a demographically connected metapopulation or is a consequence of the large size of demographically independent populations (Gagnaire et al., 2015; Lowe & Allendorf, 2010). In addition, large population sizes reduce the effect of genetic drift and increase the likelihood that any differentiation between such populations has arisen due to local selective pressures (Nielsen et al., 2009). This is because the efficiency of selection scales up with population size; therefore, the counterbalancing effect of selection is expected to be greater in broadcast marine species (Allendorf, Hohenlohe, & Luikart, 2010; Gagnaire et al., 2015). Weak genetic differentiation between such populations could therefore not only imply demographic independence, but also contrasting patterns of evolutionary resilience due to local adaptation and adaptive divergence (Allendorf et al., 2010; Gagnaire et al., 2015). Adaptive divergence is unlikely to be detected in conventional genetic data sets of only tens of loci (Leinonen, O'Hara, Cano, & Merilä, 2008; Stephan, 2016). On the other hand, methods that generate genomewide data sets (e.g., RAD-seq or ddRAD-seq) provide effective solutions for detecting loci associated with adaptive divergence, especially for species with no reference genomes (Catchen et al., 2017; Pardo-Diaz, Salazar, & Jiggins, 2015). In addition, the analytical integration of genomic and environmental data (i.e., landscape or seascape genomics) offers a framework for detecting candidate markers underlying adaptation to environmental heterogeneity (Manel & Holderegger, 2013). Here, we use populations of abalones spanning an environmentally heterogeneous longitudinal region to test the possibility that selection may be more efficient than drift in opposing the homogenizing effects of migration.

Abalones are commercially important broadcast spawning marine gastropods that are relatively abundant in tropical and temperate waters of the world (McShane, 1992). Broadcast spawners often have metapopulations with large effective sizes and high dispersal potential (Teske, Sandoval-Castillo, Waters, & Beheregaray, 2017) expected to show nil or weak genetic differentiation across vast spatial areas. However, broadcast spawners are often not demographically panmictic throughout their ranges (Selkoe & Toonen, 2011). Mounting evidence suggest that broadcast spawners show genetic differentiation associated with variation in environmental factors such as coastal topography (Banks et al., 2007), habitat availability (Selkoe, Gaggiotti, Bowen, & Toonen, 2014) and ocean currents (Piggott, Banks, Tung, & Beheregaray, 2008; Teske, Sandoval-Castillo, van Sebille, Waters, & Beheregaray, 2015, 2016). Our target species, the greenlip abalone (*Haliotis laevigata*), is a benthic reef gastropod from southern mainland Australia and northern Tasmania (Hart, Fabris, Strain, Davidson, & Brown, 2013). It is found in patches of suitable habitat forming relatively large populations that occupy hundreds of square metres with highly variable densities (e.g., 1–10 individuals per m^2; Mellin, Russell, Connell, Brook, & Fordham, 2012; Hart, Fabris, Strain et al., 2013). The high economic importance and a decline in fisheries abundance for greenlip abalone have raised interest in the development of restocking and stock enhancement programmes for the species (Hart, 2015). In southeastern Australia, neutral genetic data indicate that the species comprises metapopulations that, despite showing self-recruitment, are connected by larval dispersal across hundreds of kilometres in a stepping-stone model of isolation by distance (Miller, Mundy, & Mayfield, 2014). Greenlip abalone is distributed along a region spanning a complex and largely longitudinal environmental gradient. The region includes distinct water masses aligned along one of the longest zonal coastal boundaries in the world (McClatchie, Middleton, Pattiaratchi, Currie, & Kendrick, 2006; Ridgway & Condie, 2004). Briefly, the Australian southwestern coast is dominated by the Leeuwin Current, a relatively strong and unique current because it has a tropical origin and flows poleward along the western coast, then eastward across the Great Australian Bight. It transports warm and low salinity waters with progressively decreasing eastward flow, creating a longitudinal oceanographic gradient from Cape Leeuwin to the eastern part of the Great Australian Bight (Ridgway & Condie,

2004). From there, the zonal current flow continues as the South Australian Current, which flows eastward transporting relative warm and high salinity waters from the Great Australian Bight to the western edge of Bass Strait (Ridgway & Condie, 2004). Oceanographic connectivity simulations and estimated connectivity based on microsatellite DNA have shown that these current systems can efficiently connect broadcast spawning fauna with moderate larval period over vast spatial regions (Teske et al., 2015, 2016). No studies have so far examined the potential role of longitudinal environmental variation in promoting adaptive divergence between marine populations.

The wide distribution, relatively large population sizes and high inferred connectivity of *H. laevigata* make it an ideal system for studying adaptive divergence associated with spatial environmental variation in the sea (i.e., to study adaptation with gene flow; Tigano & Friesen, 2016). Here, we integrate genomewide data and environmental mapping within a seascape genomics framework to assess the relative influence of spatial distance and natural selection on the genetic structure of a broadcast spawner. Notwithstanding the strong oceanographic connectivity in the region, we hypothesize that selection imposed by environmental heterogeneity along southern Australia's zonal coastal boundary will drive adaptive divergence among populations of greenlip abalone. Our study generated two scenarios of metapopulation connectivity (i.e., based on putatively neutral and adaptive data sets) that provided distinct but complementary information which was recently embraced by fisheries managers (Hart, Strain, Hesp, Fisher, & Webster, 2016). As such, it exemplifies a case study that bridges the gap between collection and application of genomic data, a topic of considerable debate in the literature (Garner et al., 2016; Shafer et al., 2015). We also identified candidate genes spatially associated with aerobic and thermal variation along the coast that have functions predicted to impact on the resilience of abalone in future environments. Our Australian-based contribution adds to recent Northern Hemisphere marine studies (Benestan et al., 2016; Pavey et al., 2015) that demonstrated the influence of heterogeneous environments in shaping ecologically relevant adaptive divergence between connected populations. As such, it challenges traditional views about the relationship between connectivity and the scale of ecological barriers in the marine realm.

2 | MATERIALS AND METHODS

2.1 | Sampling and molecular methods

Foot tissue was collected from 371 greenlip abalone across 13 localities spanning most of the Western Australian greenlip abalone fishery (~29 individual per locality, Figure 1, Table 1). At all localities, 35 greenlip abalone of approximately the same age class (mature animals, 140–160 mm shell length) were collected from within 100 m of each other. Abalones were collected via commercial fishing practices and immediately processed on board a research vessel. Samples were preserved in 90% ethanol and further stored at −20°C until DNA extraction. Genomic DNA was extracted using a modified salting-out protocol (Sunnucks & Hale, 1996). Double-digest restriction site-associated DNA (ddRAD) libraries were constructed following a protocol modified from Peterson, Weber, Kay, Fisher, and Hoekstra

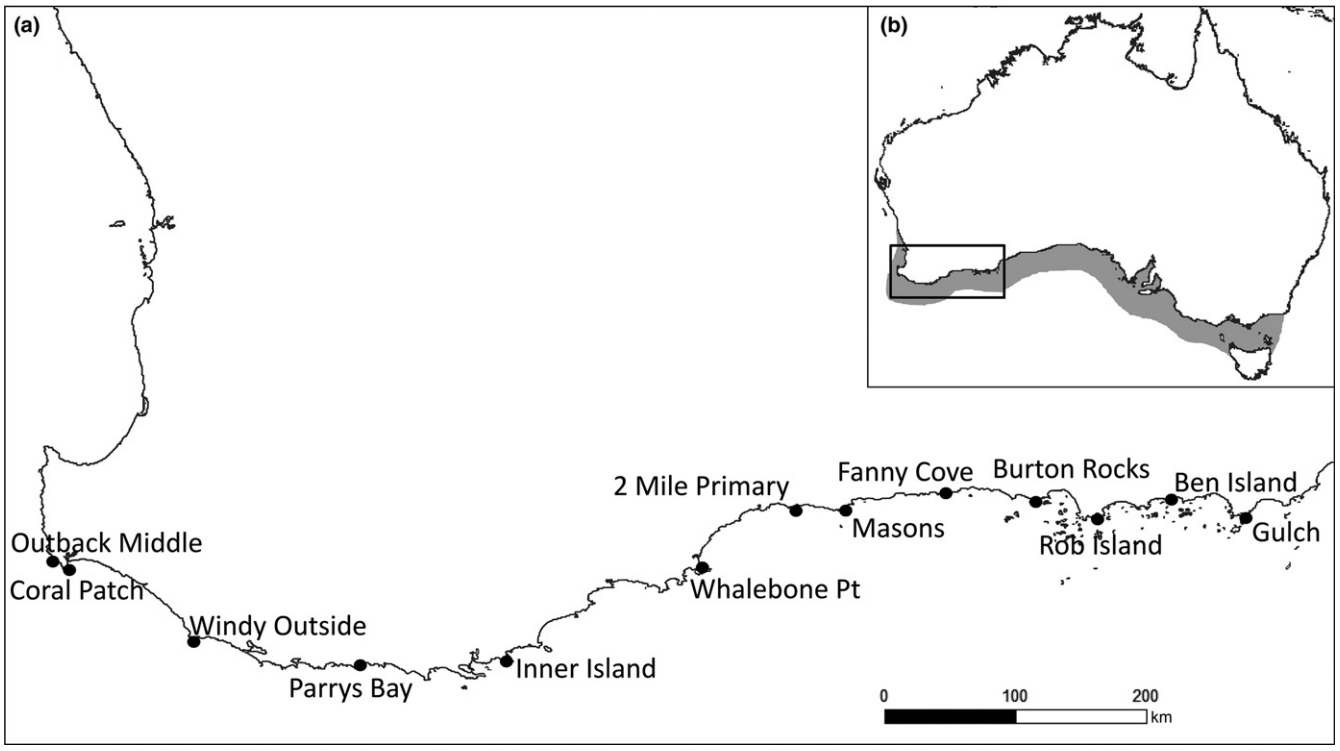

FIGURE 1 Map showing the sampling localities (a). Inset (b) shows the region targeted in this study in relation to the entire range of greenlip abalone (*Haliotis laevigata*)

TABLE 1 Levels of genetic diversity for the greenlip abalone *Haliotis laevigata* from 13 localities based on 8,786 neutral and 323 candidate adaptive SNPs

		H_E		H_O		% PL			
Site	*N*	Neutral	Adaptive	Neutral	Adaptive	Neutral	Adaptive	Longitude	Latitude
Outback Middle (OM)	29 (28)	0.249	0.264	0.262	0.285	49.0	43.7	115.067	−34.319
Coral Patch (CP)	28 (27)	0.254	0.244	0.265	0.257	48.9	44.0	115.178	−34.379
Windy Outside (WO)	29 (22)	0.280	0.240	0.312	0.232	49.2	41.2	116.035	−34.873
Parrys Bay (PB)	29 (27)	0.256	0.322	0.286	0.360	50.8	52.9	117.181	−35.033
Inner Island (II)	29 (28)	0.252	0.346	0.267	0.380	49.9	53.9	118.174	−35.003
Whalebone Pt (WP)	29 (28)	0.270	0.327	0.328	0.384	50.3	48.9	119.528	−34.364
2 Mile Primary (2MP)	28 (27)	0.261	0.369	0.293	0.433	49.7	51.7	120.170	−33.972
Masons (MS)	29 (28)	0.259	0.361	0.261	0.376	49.7	54.2	120.506	−33.972
Fanny Cove (FC)	28 (27)	0.287	0.243	0.374	0.256	52.2	51.1	121.200	−33.856
Burton Rocks (BR)	29 (28)	0.271	0.255	0.322	0.264	51.8	43.3	121.819	−33.909
Rob Island (RI)	28 (26)	0.263	0.285	0.288	0.318	50.2	46.1	122.238	−34.035
Ben Island (BI)	29 (29)	0.248	0.313	0.246	0.335	50.5	46.1	122.751	−33.899
Gulch (GL)	28 (24)	0.269	0.326	0.274	0.336	48.3	46.7	123.259	−34.023
Maximum	29 (29)	0.287	0.369	0.374	0.433	52.2	54.2		
Mean	28.6 (26.8)	0.263	0.300	0.291	0.324	50.0	48.0		
Minimum	28 (22)	0.248	0.240	0.246	0.232	48.3	41.2		

N, sample size (sample size after removing samples with more than 15% missing data); H_e, expected heterozygosity; H_o, observed heterozygosity; PL, percentage of polymorphic loci.

(2012). Briefly, 500 ng of genomic DNA was digested per sample using the restriction enzymes SbfI-HF and MseI (New England Biolabs), and one of 48 unique 6 base pair barcodes was ligated to each individual library. Replicates of 13 samples (one per locality) were included to estimate sequencing and genotyping errors. Libraries were pooled in groups of 12 samples, and then, fragments between 300 and 800 bp were selected using a Pippin Prep (Sage Science). To reduce PCR artefact bias, each pool was amplified in two 25 μl reactions using 15 PCR cycles. The two reactions per pool were combined, and the size distribution of products was confirmed using a 2100 Bioanalyzer (Agilent Technologies) and quantified using real-time PCR. Libraries were pooled in equimolar concentrations in groups of 48 samples (four pools of 12 samples, one pool of 48 samples), and each pool was sequenced on a lane of Illumina HiSeq 2000 (100 bp paired-end) at the McGill University and Genome Quebec Innovation Centre.

Reads with ambiguous barcodes or restriction sites were discarded from the data set (allowing for a maximum of two mismatches), and remaining reads were trimmed to 78 bp by removing the barcode, the restriction site and the last 10 bp using the process_radtags.pl program in STACKS 1.19 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). The DDOCENT 1.0 pipeline (Puritz, Hollenbeck, & Gold, 2014) was then used to remove low-quality bases (Phred quality score threshold of 30). DDOCENT was also used to construct a de novo assembly of putative RAD reads using a minimum depth of 15× and maximum of eight mismatches to form reference contigs. To merge putative ddRAD loci, the reference contigs were clustered based on an 80% similarity threshold. Finally, the quality-filtered trimmed reads were mapped to the reference contigs and alignment files were generated for each individual using default parameters.

FREEBAYES (Garrison & Marth, 2012), a Bayesian-based variant detection approach, was used to detect putative single nucleotide polymorphisms (SNPs) from the aligned reads of all individuals. To minimize the retention of false SNPs due to sequencing error, SNPs which were called in 80% of the individuals, with minimum depth of coverage of 5× per individual, a minimum allele balance of 20% at heterozygous genotypes and a minimum minor allele frequency of 0.03 across the data set were selected (Table 2). In an attempt to avoid linked markers, the best quality SNP per contig was chosen for inclusion in the data set using a custom perl script. The resulting vcf file was converted to other program-specific input files using PGDSPIDER 2.0.5.1 (Lischer & Excoffier, 2012). Finally, individuals with more than 15% missing data were removed using a custom script.

2.2 | Categorizing neutral and candidate adaptive loci

The contribution of natural selection to the overall pattern of genetic differentiation was conservatively assessed by combining the results of two F_{ST} outlier approaches. First, we used the method proposed by Beaumont and Nichols (1996) as implemented in LOSITAN (Antao, Lopes, Lopes, Beja-Pereira, & Luikart, 2008). This method uses a Bayesian approach to model the expected distribution of the relationship between F_{ST} (Wright's fixation index) and H_e (expected heterozygosity) under an island model of migration with neutral markers. The expected distribution is compared to the observed distribution to identify outlier loci that have excessively high F_{ST} and

TABLE 2 Number of SNP retained after each filtering step for the greenlip abalone *Haliotis laevigata*

Step	SNP count
Raw SNP catalogue	604,394
Genotyped in	
80% of individuals, base quality ≥30, minor allele frequency >0.03	178,989
Sequencing errors, paralogs, multicopy loci and artefacts of library preparation	
(1) Allele balance (>20% and <80%)	68,570
(2) Mapping alleles quality ratio (>0.8 and <1.2)	55,360
(3) Paired reads (loci supported by forward and reverse reads)	48,115
(4) Read quality (ratio quality/coverage depth >0.2)	38,751
(5) Read depth (≤mean depth + (2*standard deviation))	38,608
(6) Present in 80% of individuals in 80% of populations	31,662
(6) Hardy–Weinberg equilibrium in >75% localities	28,137
(7) Bi-allelic and single SNP per locus	9,109
Outlier detection	
BAYESCAN outliers	357
LOSITAN outliers	408
Outliers identified with both methods	323
Genotype–environment association	
gINLAnd	58
Putatively neutral	8,786

are therefore potentially under selection. The neutral distribution was simulated with 10,000 iterations at the 99.9% confidence level.

Second, we used BAYESCAN 2.1 (Foll & Gaggiotti, 2008), which identifies outlier loci with a Bayesian test based on a logistic regression model that decomposes F_{ST} values into a locus-specific component (α, selection effect) and a population-specific component (β, demographic effect). For each locus, BAYESCAN was used to apply a reversible jump Markov chain Monte Carlo algorithm to explore two models, one with and one without α, and then determine whether selection is necessary to explain the observed pattern of diversity at that locus. Twenty pilot runs of 5,000 iterations were used to estimate the distribution of α-parameters, followed by 100,000 iterations for sampling. Outlier loci were identified using a 5% false discovery rate with a prior odd of 10. Outlier loci detected by both LOSITAN and BAYESCAN were conservatively selected as candidate adaptive loci. Subsequent analyses of population genetic structure and seascape genomics (listed below) were run for the candidate adaptive and neutral data sets separately.

2.3 | Genetic diversity, population structure and genetic connectivity

Genetic diversity within each locality sample was assessed as mean nucleotide diversity (π), mean expected heterozygosity (H_e) and percentage of polymorphic loci using ARLEQUIN 3.5 (Excoffier & Lischer, 2010). Pairwise F_{ST} values were also estimated in ARLEQUIN, with their significance assessed with 1,000 permutations.

Population structure was examined using model-based and model-free approaches. First, a Bayesian clustering algorithm implemented in FASTSTRUCTURE (Raj, Stephens, & Pritchard, 2014) was used. FASTSTRUCTURE defines populations by minimizing departures from Hardy–Weinberg equilibrium and maximizing linkage equilibrium, using a variational Bayesian inference to infer the ancestry proportion of the populations in the model. Ten replicates were run for each *K* value (*K* varied from 1 to 13) using the simple prior model. The most likely number of clusters was estimated base on the optimal model complexity ($K^*\varepsilon$) calculated with the utility "chooseK.py" in FASTSTRUCTURE (Raj et al., 2014). A discriminant analysis of principal components (DAPC) was also applied using the R package ADEGENET 1.4 (Jombart & Ahmed, 2011). This multivariate method defines a system with synthetic variables in which the genetic variation is maximized between clusters and minimized within clusters. It uses *K*-means of principal components and Bayesian information criterion to determine the best-supported number of clusters. The main advantages of DAPC are its computational efficiency and lack of assumptions about population genetic models (Jombart & Ahmed, 2011).

We tested whether any inferred signal of population structure followed a model of isolation by distance (IBD) by assessing correlation between geographic and linearized genetic ($F_{ST}/1 - F_{ST}$) distance matrices using a Mantel test in GENODIVE 2 (Meirmans & Van Tienderen, 2004). Geographic distances were estimated as the shortest distance between localities following the coastline (i.e., coastal distance) using ARCMAP 10.1 (ESRI 2012). We attempted to infer genetic connectivity using a clinal model of gene flow that indirectly estimates dispersal distance (Sotka & Palumbi, 2006). This model assumes that the balance between dispersal and selection determines the geographic width of a stable genetic cline. Linkage disequilibrium (LD) integrates selection across multiple life stages and generations; therefore, when selection is not quantified, the degree of LD between loci can be used as proxy for selection to quantify dispersal distance (Sotka & Palumbi, 2006). Average pairwise LD was calculated separately for neutral and candidate loci using VCFTOOLS (Danecek et al., 2011). Clines in allele frequency for each of the 323 candidates and 500 randomly selected neutral loci were calculated using the R package HZAR (Derryberry, Derryberry, Maley, & Brumfield, 2014), with six chains each of 100,000 interaction after 10,000 interaction of burn-in. Five models were generated for each locus (Appendix S1: Table S1), and the best fit was selected using Akaike information criterion (AIC); to explore more strict criteria, we also used an arbitrary −7 log-likelihood cut-off. Individual dispersal distance per generation was estimated using the cline width from best fit model and the average LD following Sotka and Palumbi (2006).

2.4 | Seascape genomics: testing for genotype × environment associations

Environmental heterogeneity across the zonal coastal boundary was assessed using calculated and in situ data from four main

oceanographic variables over the last 100 years obtained from the NOAA World Ocean Database (Boyer et al., 2013): sea surface temperature (°C), oxygen concentration (mg/L), pH and nutrient concentration (μM). Gridded maps at 0.1° resolution were generated using the DIVA algorithm in ODV 4 (Schlitzer, 2015) to obtain annual and seasonal averages for each variable, and annual maximum and minimum sea surface temperature. Seasonal averages for all variables during spring and summer were used because these represent the peaks of spawning and settlement activity of greenlip abalone (Rodda, Keesing, & Foureur, 1997). Thus, considering these averages, we extracted information from 14 environmental data sets (listed in Appendix S1: Table S2). ARCMAP 10.1 (ESRI 2012) was used to extract oceanographic information from the gridded maps for each sampling locality. Ecological distance matrices were estimated as the pairwise difference between sampling sites for each oceanographic variable (Appendix S1: Table S2). Environmental variation and divergence between sampling sites were assessed using principle component analysis (PCA) in the R package FACTOMINER 1.25 (Lê, Josse, & Husson, 2008), with the optimal number of environmental clusters determined based on Ward's criterion.

The effects of seascape heterogeneity were explored with univariate and multivariate genotype–environment association methods to identify associations between genetic and environmental variation. First, we used a multiple regression of distance matrices (MRDM) with linearized F_{ST} values ($F_{ST}/1 - F_{ST}$) as dependent variable and six ecological distance matrices as independent variables. Because of the high collinearity expected between annual and seasonal averages, we ran this analysis using only the annual average of all four variables plus the annual maximum and minimum sea surface temperatures (Table 3). MRDM was performed using the R program ECODIST 1.2.9 (Goslee & Urban, 2007) with regression models calculated using the no parametric Spearman correlation with 1,000 permutations. Coastal distance (calculated as above) was included as an independent variable to control for IBD. We then performed stepwise selection using the R package MASS 1.3 (Venables & Ripley, 2002) to find the simplest model that adequately fits our data based on AIC (Venables & Ripley, 2002).

Second, we used a spatially explicit generalized linear mixed-model implemented in gINLAnd (Guillot, Vitalis, le Rouzic, & Gautier, 2014). For each locus, gINLAnd uses Bayesian inference to select between two competing models: one in which an environmental variable has an effect over the allele frequency and a reduced model in which the environmental variable has no effect. This approach accounts for spatial autocorrelation due to population history by including a random spatial effect calculated based on geographic coordinates. For abalone, using standard geographic coordinates provides a distorted measure of biological distance among sites because this would connect sites via land distances. To overcome this, we calculated new coordinates using multidimensional scaling projections of coastal distances between sampling sites using the R package MASS 1.3 (Venables & Ripley, 2002). Using these coordinates and a subset of 500 randomly selected loci, we inferred parameters of the special covariance to control for spatial genetic structure in the final models. We ran this analysis using the annual average of all four variables, plus the annual maximum and minimum sea surface temperatures (Table 3). To control for false positives, we conservatively selected as under selection only those loci that showed a gINLAnd log Bayes factor >20.

TABLE 3 Multiple regressions on distance matrices showing the correlation of greenlip abalone genetic distances with ecological distances

	Full model		Reduced model		Independent	
Variable	***b***	***p***	***b***	***p***	R^2	***p***
Nutrients concentration	0.0702	.558	0.1800	.096	.0678	.059
Oxygen concentration	**0.5502**	**.002**	**0.4929**	**.003**	**.1734**	**.008**
pH	−0.1835	.220			.0085	.435
Average temperature	−0.6193	.053	−0.2690	.073	.0127	.249
Minimum temperature	**0.3629**	**.014**	**0.3248**	**.018**	**.1599**	**.011**
Maximum temperature	0.1056	.393			.0052	.534
Coastal distance	0.4189	.092			.0396	.078
	R^2	*p*	R^2	*p*		
Model	.3963	**.019**	.3513	**.013**		

Included are the full model (all oceanographic variables) and a reduced model (best Akaike information criterion). Significant values are in bold ($p < .05$). Coastal distance was included to control for geographic effects.

2.5 | Functional annotation

To examine functions putatively associated with the ddRAD loci, we initially used BLAST+ v2.2.28 (Camacho et al., 2009) to perform a BLASTN search with an E-value threshold of 1×10^{-05}. The flanking sequences of each SNP were compared against four currently available *Haliotis* transcriptomes: *H. midae* (Franchini, Van der Merwe, & Roodt-Wilding, 2011), *H. rufescens* (Wit & Palumbi, 2013), *H. laevigata* (Shiel, Hall, Cooke, Robinson, & Strugnell, 2015), and *H. tuberculata* (Harney et al., 2016). The results were annotated against the UniProtKB/Swiss-Prot database (Consortium, 2014) with an E-value threshold of 1×10^{-03}. We then tested whether the 323 candidate loci were enriched for specific functional categories by performing a gene ontology term analysis in TOPGP v2.24 (Alexa & Rahnenfuhrer, 2010). We used Fisher's exact test with a minimum node size of five and the full annotated data set (neutral and candidates loci) as specific background gene set coverage, as recommended by Alexa and Rahnenfuhrer (2010). In addition, the annotated candidate loci were manually categorized into several broad functional categories by searching for information in the relevant literature.

3 | RESULTS

3.1 | SNP genotyping

Approximately 3 billion raw sequence reads were obtained from the eight lanes of Illumina. After filtering and trimming, the final data set contained ~1.6 billion reads of 78 bp each. From these, ~604,000 ddRAD loci were identified. The data set was reduced to 28,137 SNPs, of which 9,109 were used for further analyses (Table 2). Fifty-one samples were removed from the final data set because they had >15% missing data; the remaining 349 samples had an average of 2.9% missing data.

3.2 | Categorizing neutral and candidate adaptive loci

LOSITAN and BAYESCAN detected 408 and 357 outliers, respectively. Of these, 323 loci (~3%) were identified by both methods and considered as candidate adaptive loci (Figure 2). Importantly, included in these 323 are also all candidate loci identified by gINLAnd (see

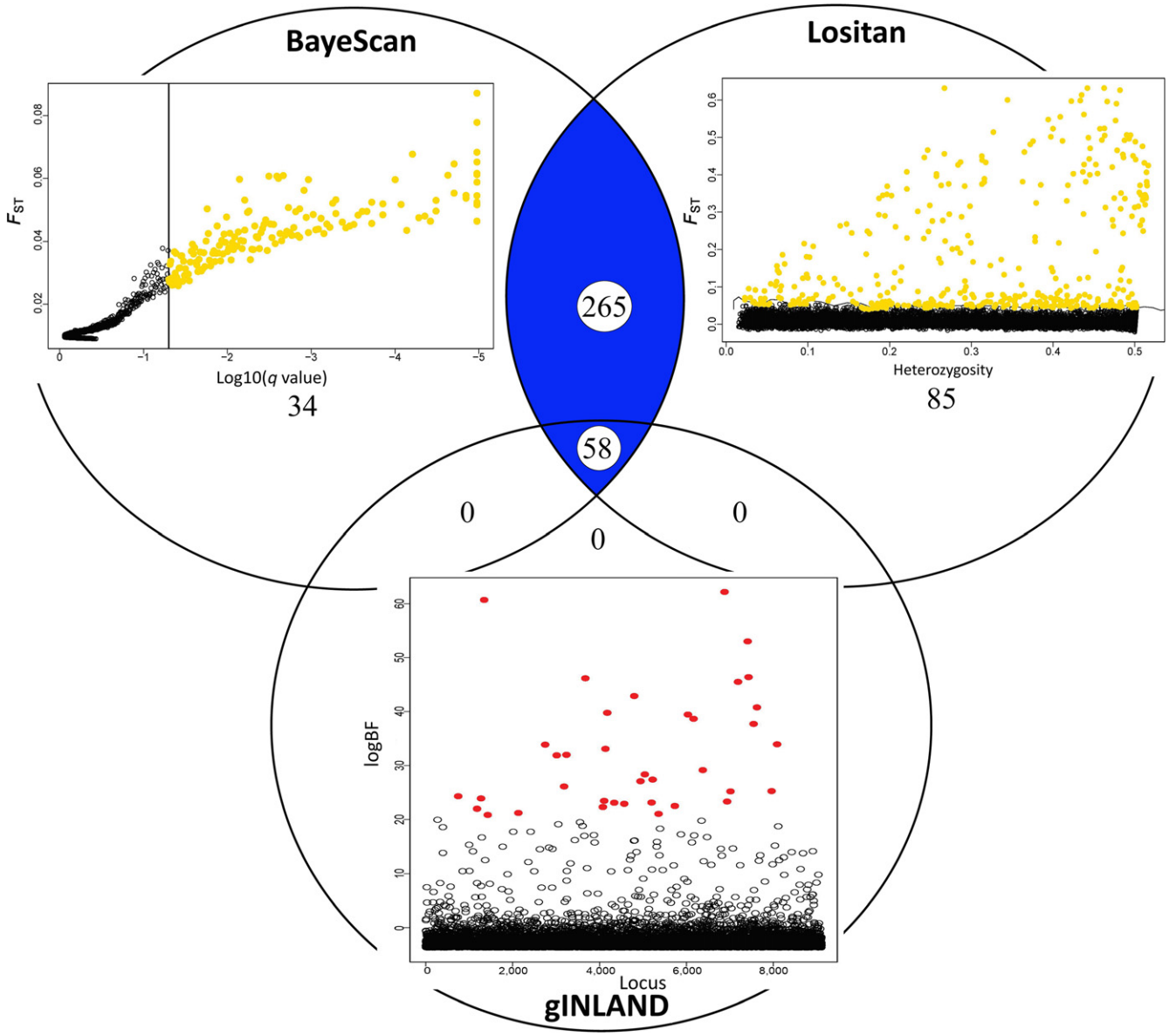

FIGURE 2 Venn diagram of loci detected as outliers by two approaches (BAYESCAN and LOSITAN) and loci associated with six environmental variables (gINLAnd). BAYESCAN: locus-specific F_{ST} plotted against the log 10 of the false discovery rate (*q* value), vertical line shows critical log10 q used to identifying outliers, and loci significant at 5% false discovery rate are indicated by yellow dots. LOSITAN: locus-specific F_{ST} plotted against expected heterozygosity, line shows the 99.9% confidence intervals used to identify outliers, and loci significant at 5% false discovery rate are indicated by yellow dots. gINLAnd: plot shows locus-specific log Bayesian factor, loci with allele frequency significantly associated with oxygen concentration are indicated by red dots (BF > 20). Blue area represents loci detected by all three methods or only by the two outlier methods; these were conservatively considered as the 323 candidate adaptive loci

below). Subsequent analyses were conducted for the candidate data set of 323 loci and for the neutral data set of 8,786 loci.

3.3 | Genetic diversity, population structure and genetic connectivity

Neutral genetic diversity was very similar across sampling localities, with percentage of polymorphic loci ranging from 48.3% at Gulch to 52.2% at Fanny Cove. Putatively adaptive diversity was also similar between localities, with polymorphism ranging from 41.2% at Windy Outside to 54.2% at Fanny Cove (Table 1).

Levels of population genetic differentiation across localities were nil or very low based on the neutral data set (global F_{ST} = 0.0081; 95% CI = 0.0074–0.0088), with only two of the 78 pairwise comparisons significantly different than zero (Appendix S1: Table S3). On the other hand, levels of differentiation based on the candidate data set were moderate to high (global F_{ST} = 0.267; 95% CI = 0.196–0.318), with 71 of the 78 pairwise comparisons significant (Appendix S1: Table S4). For the neutral data set, both model-based and model-free clustering methods indicated the presence of a single population across the study area (Figure 4a; Appendix S1: Figures S1A and S2A, S3). For the candidate data set, the same methods indicated five distinct population clusters across the study area (Figures 3 and 4b; Appendix S1: Figures S1B, S2B), a result consistent with F_{ST} analyses of this data set. These are found in the Augusta region (Outback to Windy Outside, cluster 1), in the Albany region (Parrys Bay and Whalebone Port, cluster 2), in the Hopetoun region (Inner Island to Mason, cluster 3), in the West Region (Fanny Cove and Burton Rocks, cluster 4) and in the Eastern Region (Rob Island to Gulch, cluster 5; Figure 6c).

The IBD analysis was significant for the neutral data set but not for the candidate data set ($p < .001$ and $p = .565$, respectively; Appendix S1: Figure S4). Regarding the indirect estimate of dispersal distances, the inferred genetic clines were significant for 186 (37.2%) out of the 500 tested neutral loci and in 316 (97.8%) of the 323 candidate loci (Appendix S1: Table S5). Under higher statistical stringency (i.e., significance with log-likelihood <−7), the equivalent results were 76 (15.2%) and 291 (90.1%), respectively (Appendix S1: Table S5). Based on statistical stringency results, the average cline width was 124.0 and 46.97 km for the neutral and candidate loci, respectively. The associated estimated average dispersal distances per generation using the clines was significantly larger for the neutral loci (25.84 km) than for the candidate loci (9.96 km; $p < .001$; Appendix S1: Table S5 and Figure S5). Overall, from a neutral perspective, the results of analyses of population structure and dispersal indicate one metapopulation of greenlip abalone in our study region connected according to a stepping-stone model (see Section 4).

3.4 | Seascape genomics

The PCA based on the oceanographic data sets (Appendix S1: Table S2) revealed four environmentally divergent regions: Augusta (from Outback to Windy Outside), Albany (Parrys Bay and Whalebone Port), Hopetoun (Whalebone Port to Burton Rocks) and Eastern (Rob Island to Gulch; Figures 5, 6b). These regions are thought to exert selective pressures that affect the distribution and connectivity of abalone and promote local adaptive divergence. In fact, the distribution of the five inferred adaptive clusters is in marked spatial congruence with the four selectively divergent regions defined based on environmental variables (Figure 6). An exception is that Inner Island clusters environmentally with Parrys Bay and Whalebone Port, whereas samples from this locality cluster genetically with those from the Hopetoun region. In addition, Fanny Cove and Burton Rocks cluster environmentally with Two Mile Primary and Mason, but genetically compose their own unique adaptive cluster.

The joint analysis of environmental and adaptive variation using MRDM found statistical support for correlations between the candidate data set and minimum sea surface temperature and oxygen concentration, even after controlling for spatial geographic distance (Table 3). This indicates that although spatial distance affects neutral genetic structure, differences in temperature and oxygen concentration between localities promote adaptive differentiation among greenlip abalone populations. This is consistent with results from the

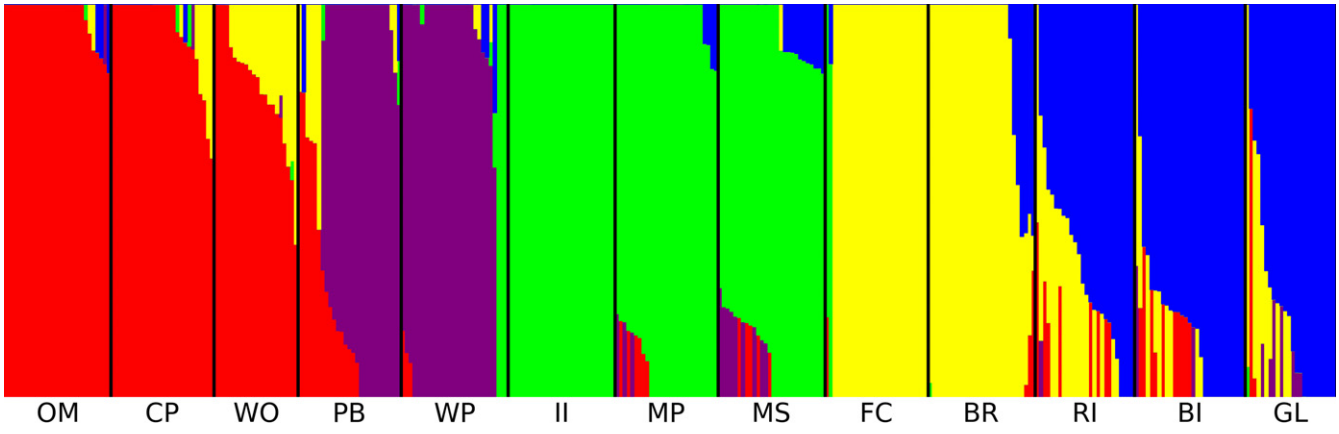

FIGURE 3 FASTSTRUCTURE clustering plots for the greenlip abalone *Haliotis laevigata* based on 323 candidate SNPs. The plot is of $K = 5$, which is the inferred number of clusters for the candidate data set using the optimal model complexity (K^*_ε; neutral data set has an inferred $K = 1$, see Appendix S1: Figures S1, S2 and S3). Thin black lines separate sampling localities

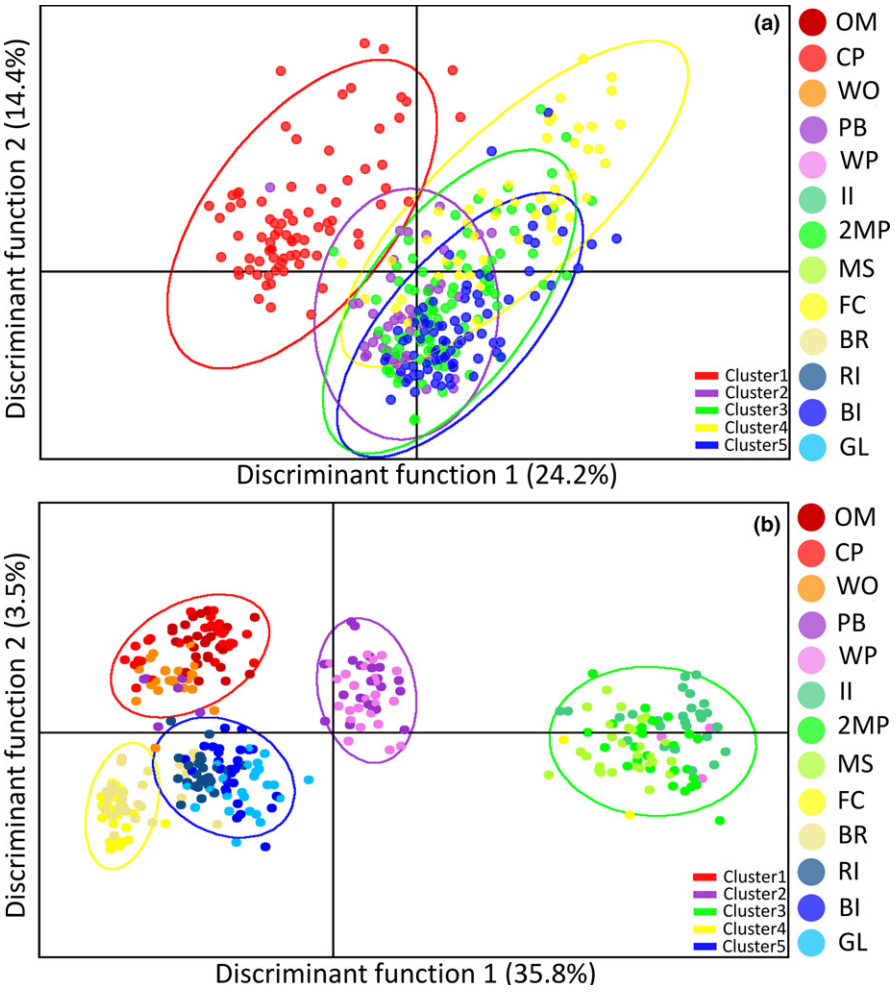

FIGURE 4 Discriminant analysis of principal components affecting genetic variation in *Haliotis laevigata*. (a) For 8,786 neutral SNPs, the first two discriminant functions explain 38.6% of the genetic variation (DF1 = 24.2%; DF2 = 14.4%). (b) For 323 candidate SNPs, the first two discriminant functions explain 39.3% of the genetic variation (DF1 = 35.8%; DF2 = 3.5%). For comparative purposes, both plots are of $K = 5$, which is the inferred number of clusters for the candidate data set using Bayesian information criterion (neutral data set has an inferred $K = 1$, see Appendix S1: Figures S1 and S2)

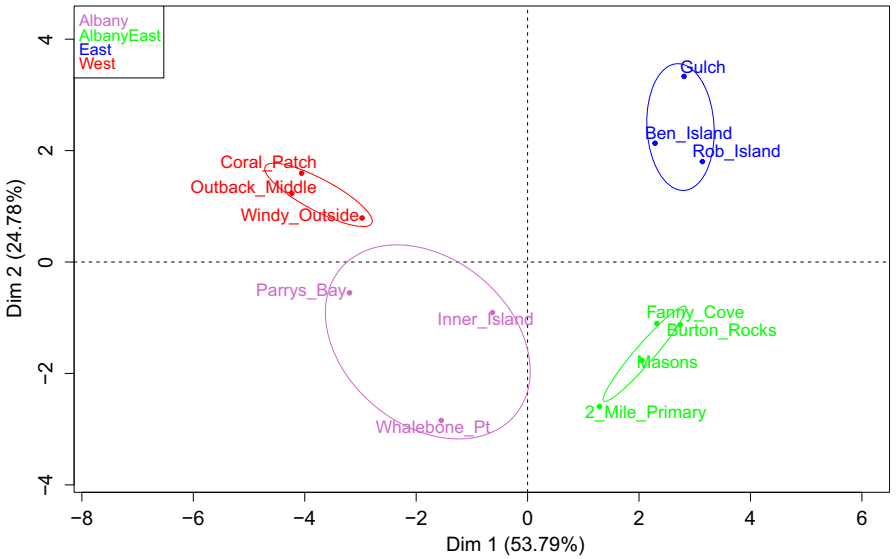

FIGURE 5 Principal component analysis based on fourteen oceanographic variables (Appendix S1: Table S2). The scatterplot shows the first two principal components, which together explain 78.6% of the variation. Dots are coloured according to the most probable environmental group. Ellipses represent the 95% confidence level of these groups

spatially explicit genotype–environment association analysis carried out in gINLAnd, which provided strong evidence for associations between allele frequencies and environmental variables at 58 loci (Appendix S1: Table S6); with oxygen concentration associated with 37 loci (Figure 2), pH with 19 and sea surface temperature with 4 loci.

3.5 | Functional annotation

Of all the 9,109 loci, 1,128 were annotated and assigned to 10,440 GO terms (Appendix S1: Table S8). For the 323 candidate loci, 95 were annotated and assigned 713 GO terms (Appendix S1: Table S7).

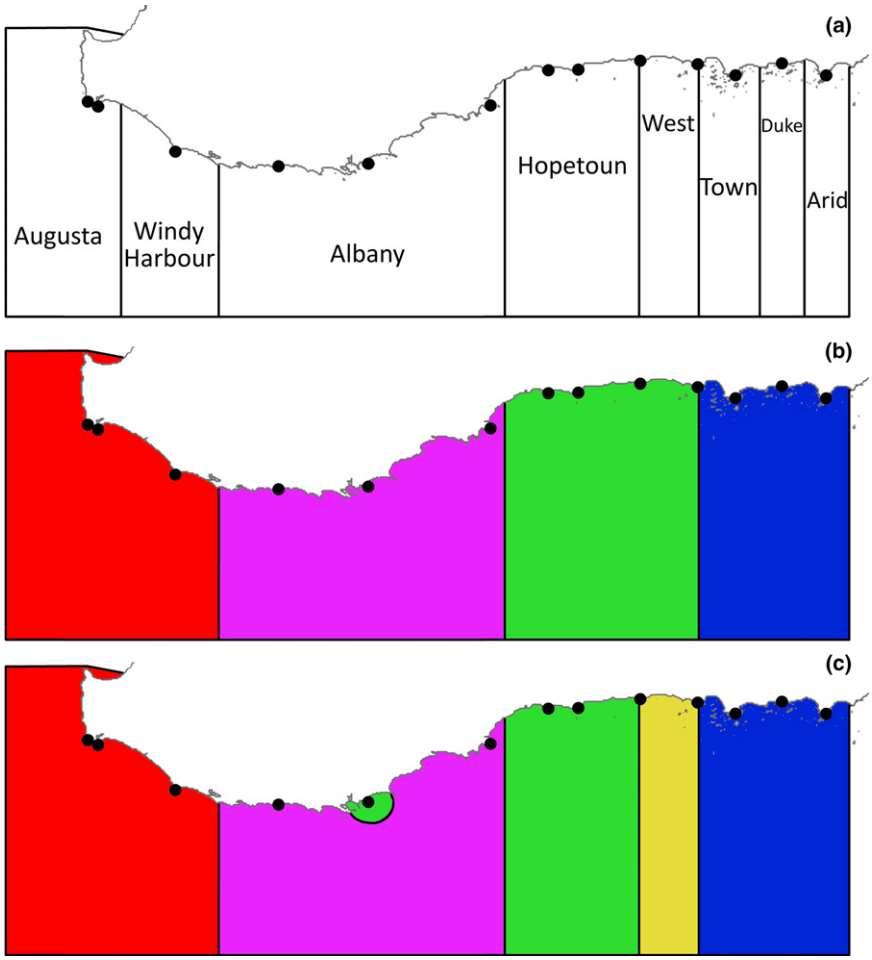

FIGURE 6 Maps showing (a) the eight fisheries management subareas for the greenlip abalone (*Haliotis laevigata*) range covered in this study; (b) the geographic distribution of the four environmental regions detected based on fourteen oceanographic variables; and (c) the geographic distribution of the five adaptive clusters of greenlip abalone detected based on 323 candidate SNPs

The candidate GO enrichment analyses identified two molecular functions and 18 biological processes that were significantly enriched ($p < .01$, Appendix S1: Table S8). Most of the genes containing SNPs under selection (73 of 95 annotated genes) could be categorized as having functions associated with cellular respiration and energetic processes; DNA repair, synthesis, transcription and translation; immune/stress response; membrane transport and homeostasis; or cell proliferation, differentiation and embryonic development (Appendix S1: Figure S6, Table S7). As discussed below, these biological processes are potentially influenced by environmental variation in oxygen and temperature.

4 | DISCUSSION

Our seascape genomic study identified and compared patterns of putatively neutral and adaptive genetic variation in a metapopulation of greenlip abalone along ~11° of longitude in coastal temperate waters of southern Australia. Neutral genetic structure was remarkably low across the study region (global $F_{ST} = 0.0081$; 95% CI = 0.0074–0.0088), and it was weakly correlated with coastal distance ($R^2 = .34$; $p < .001$). On the other hand, model-based and model-free approaches using a candidate loci data set revealed five putatively adaptive clusters largely congruent with oceanographic regions discriminated by environmental PCA. Adaptive genetic divergence was associated with differences in oxygen concentration and minimum temperature between these regions. In addition, several candidate loci were annotated to genes whose functions putatively affect high temperature and/or low oxygen tolerance. Our results indicate that spatially varying selection due to coastal seascape heterogeneity maintains adaptive divergence in a genetically connected metapopulation of a broadcast spawner. Although these conclusions challenge traditional views about the relationship between gene flow and adaptation, they are consistent with recent theoretical models and empirical evidence showing that local adaptations can be maintained despite high gene flow (reviewed in Tigano & Friesen, 2016). As discussed below, our study also bridges the gap between collection and application of genomic data, a topic of considerable debate in the literature (Garner et al., 2016; Shafer et al., 2015).

4.1 | Connectivity and adaptive divergence along an environmentally heterogeneous seascape

Knowledge about population structure and dispersal across species ranges is valuable to understand how different sources of adaptive variation (e.g., standing genetic variation, new mutations or adaptive introgression) might interact with gene flow when shaping local patterns of adaptive divergence (Tigano & Friesen, 2016). Our findings

from the neutral data set indicate one well-connected metapopulation of greenlip abalone. The average scale of dispersal estimated with an indirect method based on genetic clines may appear small (25.8 or 38.5 km depending on statistical stringency, Appendix S1: Table S5) compared to the low genetic structure inferred across the ~800 km long study region. However, several of our estimated spatial genetic clines (i.e., geographic zones in which populations interbreed; Sotka & Palumbi, 2006)) were much longer than the average cline width of 124 km used to estimate average dispersal distance. For instance, 14 of 76 clines that received highest statistical support were longer than 400 km (Appendix S1: Figure S5), supporting events of dispersal above that spatial scale. Thus, the overall neutral structure of greenlip abalone across southwestern Australia appears to be influenced by both fine- and large-scale dispersal consistent with a stepping-stone model of IBD. Genetic connectivity facilitated by stepping-stone dispersal has been reported in numerous marine species with larval dispersal (Crandall, Treml, & Barber, 2012; Ellis, Hodgson, Daniels, Collins, & Griffiths, 2017; Pinsky, Montes, & Palumbi, 2010; Wood, Paris, Ridgwell, & Hendy, 2014), including in a microsatellite study of greenlip abalone in southeastern Australia that provided evidence for local recruitment as well as stepping-stone connectivity over hundreds of kilometres (Miller et al., 2014). In fact, the integration of stepping-stone theory substantially increases the spatial scale of modelled advection connectivity in marine broadcast spawners (Teske et al., 2015). Additionally, our dispersal estimates are averaged per generation and therefore do not rule out the possibility that events of long-distance dispersal help maintaining connectivity over large scales. For instance, Piggott et al. (2008) used microsatellite data, migration simulations and oceanographic particle dispersal modelling for the abalone *Haliotis coccoradiata* over ~1,000 km of the east coast of Australia. They showed that the inferred signal of high local larval retention (within 20 km scale) needs to be combined with a low proportion of long-distance gene flow (100s km scale) to account for the observed broadscale weak genetic differentiation. For southwestern Australia, if we consider only the periods relevant for the spawning and pelagic larval duration of greenlip abalone (Prince, Sellers, Ford, & Talbot, 1987; Shepherd, Lowe, & Partington, 1992), advection connectivity simulations suggest that >80% of larvae released along our study region will reach the boundary currents responsible for long-distance transport (see supplementary material in Teske et al., 2015). Indeed, population genetic studies of intertidal organisms with contrasting life histories (e.g., kelps and snails) have shown that strong oceanographic currents along this coastal region can promote eastward dispersal from Cape Leeuwin towards the Great Australian Bight (Coleman, Feng, Roughan, Cetina-Heredia, & Connell, 2013; Teske et al., 2017).

In terms of adaptive divergence, the relatively large size of local populations of greenlip abalone (Hart, Fabris, Strain et al., 2013; Mellin et al., 2012) and of its broader connected metapopulation are expected to drive population responses to ecologically mediated selection (Allendorf et al., 2010; Kawecki & Ebert, 2004; Yeaman & Whitlock, 2011). This hypothesis is supported by the high and significant genetic divergence detected with the candidate adaptive loci (Figures 3 and 4b), consistent with the view that selection can drive adaptive divergence and reduce homogenizing effects of gene flow in particular locations of the genome (Bradbury et al., 2013; Nosil, Funk, & Ortiz-Barrientos, 2009). The lack of IBD (Appendix S1: Figure S4B) and the strong signal for genotype–environment associations after controlling for spatial structure and distance with the candidate data set (Figure 2, Table 3; Appendix S1: Figure S7, Table S6) support the idea that divergence is driven by the environment rather than geography. While geographic proximity between localities would generally be associated with similar oceanographic conditions, similar conditions can also be found between geographically distant localities. For example, our sample from Rob Island is oceanographically more similar to Gulch (a site ~90 km away) than to Burton Rocks (~40 km away).

Some studies have attributed findings of heterogeneous divergence along the genome to various other processes rather than selection, but this is unlikely in our study system. One such process is allelic surfing (i.e., when the frequency of a novel neutral mutation gradually increases with the wave of a range expansion), which can mimic the selection signature of outlier tests (Excoffier & Ray, 2008; Klopfstein, Currat, & Excoffier, 2006). However, because the probability of allelic surfing is inversely proportional to migration rates and carrying capacity (Excoffier & Ray, 2008; Klopfstein et al., 2006), allelic surfing appears unlikely in greenlip abalone because of its high connectivity and population densities (Hart, Fabris, Strain et al., 2013; Mellin et al., 2012). Another possibility, proposed by Bierne, Welch, Loire, Bonhomme, and David (2011) is that "neutral" barriers to gene flow can couple with "adaptive" barriers and produce patterns of genetic structure unrelated to ecology that originated due to historical or contemporary "neutral" barriers. The remarkably low levels of metapopulation structure are inconsistent with the presence of "neutral" barriers; this is anticipated given the lack of geological or oceanographic features in our study region that could have promoted biogeographic structure in planktonic dispersers (reviewed in Teske et al., 2017). Finally, sweepstakes in reproductive success can create temporal patterns of genetic variation that are not necessarily adaptive (Hedgecock & Pudovkin, 2011). However, these patterns tend to be chaotic, while the genetic structure found in this study of greenlip abalone appears environmentally ordered. We hypothesize that the main source of adaptive variation for the greenlip abalone metapopulation originates from genetic variants already segregating locally (i.e., standing variation), which interact with stepping-stone mediated gene flow when shaping local patterns of adaptive divergence.

Genotype–environment association methods have successfully detected selection due to environmental heterogeneity in a range of simulated and empirical studies (De Mita et al., 2013), including when adaptive traits are polygenic and population structure is complex (Brauer et al., 2016; Villemereuil, Frichot, Bazin, François, & Gaggiotti, 2014) and when populations have undergone geographic expansion (Frichot, Schoville, de Villemereuil, Gaggiotti, & François, 2015). Genotype–environment associations have been described for marine species, including abalone (Benestan et al., 2016; Riginos &

Liggins, 2013; Wit & Palumbi, 2013). For instance, Wit and Palumbi (2013) used transcriptome-derived SNPs to report associations between population structure in green abalone (*H. rufescens*) and temperature gradients along the coast of California. In greenlip abalone, the distribution of putatively adaptive variation was strongly associated with selection linked to minimum sea surface temperature and oxygen concentration. As temperature affects essentially all cellular process, and oxygen concentration is important for all aerobic metabolic process (and influences thermal tolerance in marine ectotherms; Clarke, 2003; Pörtner, 2010; Sanford & Kelly, 2011), we would expect these to be key selective factors affecting marine organisms. Empirical evidence from other studies supports the link between fitness traits in our nonmodel study system with the environmental context in which candidate loci were selected. Harris, Maguire, Edwards, and Johns (1999) showed that variations as small as 1 mg/L in oxygen concentration affect growth and survival of greenlip abalone. Differences in optimal temperatures for growth and critical maximum temperature have been reported between greenlip abalone originating from warmer (Stone et al., 2014) and cooler waters (Gilroy & Edwards, 1998). Likewise, gene expression profiles in greenlip abalone have been found to be predictors of resilience to summer mortality that occurs in association with raised temperature and lowered oxygen levels (Shiel, Hall, Cooke, Robinson, Stone et al., 2017; Shiel, Hall, Cooke, Robinson, & Strugnell, 2017). Our results suggest that such fitness differences reported for greenlip abalone populations are due to genetic adaptations associated with local temperature and oxygen availability occurring at small spatial scales.

4.2 | Function of candidate genes

Although the association of candidate genes with environmental variables does not necessarily imply causality or spatial differences in fitness performance, an understanding of gene function contributes to refine views about patterns of selection and organismal performance along the zonal marine environment targeted in this study. A relatively large number of candidate loci were annotated as genes that influence tolerance to high temperatures or low oxygen concentrations (Appendix S1: Figure S6, Tables S7 and S8). This is compatible with our proposal of the existence of locally adapted clusters in the greenlip abalone. Information about the functions of these genes across species, including the environmental context in which the genes function in other abalone species, provide useful leads for future research in this commercially important group. More broadly, such information could help us test hypotheses about the repeated use of the same genes during adaptive evolution (Conte, Arnegard, Peichel, & Schluter, 2012).

The foot or pedal muscle makes up more than 60% of the wet weight of an abalone, and it is likely that aerobic energetic processes affecting the ability of the animal to grip or move using the foot muscle, especially under conditions of high temperature and low oxygen, would affect abalone survival in the wild. Differences in aerobic capabilities (metabolic efficiency per unit of oxygen available), both between and within abalone species, have been found to depend on their biogeography (Baldwin, Elias, Wells, & Donovan, 2007; Dahlhoff & Somero, 1993). Genetic variants associated with such physiological differences could affect the ability of individuals to survive at low oxygen levels and therefore be under strong selective pressure. Among the annotated candidate loci, 13 were categorized as having functions involved in aerobic metabolism, ATP energetics, glycolysis or energy processes. One candidate locus is located in the cytochrome c oxidase (COX) gene, which plays a key role in mitochondrial aerobic respiration (Li, Park, Deng, & Bai, 2006). This gene affects the immune response of abalones (van Rensburg & Coyne, 2009) and has been found to influence thermal acclimation in two species of abalone by adjusting metabolism in order to meet higher oxygen demands at high temperatures (Dahlhoff & Somero, 1993). Another candidate locus was annotated as the heme α-synthase gene, an important component of the COX enzyme (Papa et al., 1998). It has been suggested heme α-synthase has an important role in the response to hypoxic conditions in animals (Druyan, Cahaner, & Ashwell, 2007; Shibahara, Han, Li, & Takeda, 2007). Adaptation to reduced oxygen levels (hypoxia) requires coordinated reduction of metabolic demand and increase of ATP production efficiency (Wheaton & Chandel, 2011). Six candidate loci mapped to genes affecting general ATP energetics. Additionally, four candidate loci were mapped to genes involved in the glycolysis process (production of ATP in anaerobic or aerobic conditions), whose efficiency plays a fundamental role in the response of mammals to low levels of oxygen and high energy demands (Robin, Murphy, & Theodore, 1984). Moreover, four enriched gene functions (nitrogen compound metabolic process, CoA-ligase activity, citrate metabolic process, dicarboxylic acid metabolic process, see Appendix S1: Table S8) are involved in glycolytic and metabolic pathways which have important roles in adaptation to hypoxia and anoxia in different animals, including molluscs (Guévélou et al., 2013; Hermes-Lima et al., 2015; Ivanina, Nesmelova, Leamy, Sokolov, & Sokolova, 2016).

Thermal and oxidative stresses diminish the integrity of nucleic acids (Evans, Dizdaroglu, & Cooke, 2004; Manova & Gruszka, 2015). Five candidate loci occurred in genes affecting DNA repair, replication and synthesis; four occurred in genes affecting translational activity and 13 occurred in genes affecting transcription of DNA. In addition, three enriched gene functions (sister chromatid cohesion, nuclear chromosome segregation, transposition; Appendix S1: Table S8) are associated with chromosome stability, chromosome replication and segregation during mitosis cell reproduction. High temperature and low oxygen stresses also make animals more vulnerable to disease influencing survival and reproduction. Eleven candidate loci were mapped to genes associated with immune and stress response. One of these candidates was located in a Pumilio homolog gene. The product of this gene functions as a post-transcriptional/translational repressor in eukaryotes and has been reported to effectively represses virus replication in infected organisms (Un Huh & Paek, 2013). High temperature promotes viral infections which have been implicated in abalone mass mortalities worldwide, including populations of greenlip abalone in Australia

(Corbeil, Williams, McColl, & Crane, 2016). Therefore, genetic variations on Pumilio homolog gene could allow the immune response of abalones to adapt to different temperatures.

4.3 | Management implications: integrated enhancement of fishery, selective breeding and metapopulation viability

The 9,109 filtered SNPs genotyped for 371 individuals (the largest population genomic data set reported so far for abalone) offers high resolution for improving management policies and decisions, which previously were being made blind with respect to patterns of adaptive genetic variation. The neutral and putatively adaptive levels of connectivity identified here provide complementary information for management (sensu Funk, McKay, Hohenlohe, & Allendorf, 2012). The neutral data set indicates that a greenlip abalone may have parents that recruited locally or may be descendants from abalones that recruited elsewhere in the vast sampled area but moved via stepping-stone dispersal. On the other hand, the candidate data set suggests that abalones that recruited within their local adaptive cluster may show higher fitness (with regard to variation in the aerobic and thermal environment) than if they recruited in clusters found elsewhere (Figure 6c). Therefore, our results indicate one connected metapopulation across the sampled area and five spatially distinct adaptive clusters.

These findings were recently utilized in two ways by the Western Australian government: first, in a successful application for MSC (Marine Stewardship Council) certification of its greenlip abalone fishery (Hart et al., 2016), and secondly in its aquaculture management policies (DoF 2016). The greenlip abalone fishery in Western Australia is in a unique developmental trajectory as it is slowly evolving from a "pure wild harvest" fishery to an integrated enhancement fishery, in which the catch is comprised of both wild and aquaculture products (Hart, 2015). The MSC accreditation process recognizes that the use of juveniles from aquaculture facilities in sea ranching and the sea ranching process per se, implicates a genetic risk to wild greenlip abalone populations (Laikre, Schwartz, Waples, & Ryman, 2010). The Western Australian authorities have therefore taken the results from our study as the genetic baseline to develop a "genetic management plan." This plan includes a monitoring programme to assess any impacts of the commercial sea ranching and restocking activities, as well as explicit policies that promote the maintenance of patterns of adaptive divergence (Daume, Gardner, Leporati, & Trott, 2017; DoF 2016; Hart et al., 2016). In particular, the "progeny diversity strategy" explicitly recommends the use of broodstock collection and breeding programmes based on the five adaptive clusters described in this study to ensure that only genetically appropriate progenies are released into the marine environment (DoF 2016). These management policies assisted the fishery to become the world's first MSC certified wild abalone fishery. As such, our study contributes a documented example about the uptake of genomic information in biodiversity management, a topic of substantial controversy in the literature (Funk et al., 2012; Garner et al., 2016; Shafer et al., 2015).

The adaptive divergence reported here appears to be underpinned by variation in seawater temperature and oxygen concentrations and should affect traits relevant for the survival, growth and reproduction of abalone. These are economically important traits and therefore the loci that appear to be under selection might have potential application as markers for selective breeding to improve the productivity of the growing abalone aquaculture industry (Yue, 2014). For instance, the candidate loci detected here might be useful for ranking and selecting breeders for improving temperature tolerance, one of the most costly environmental problems confronted by the abalone mariculture industry in Australia (Hooper et al., 2014).

The Australian abalone fishery, which represents a large proportion of the wild abalone catch around the world, has shown signs of decline in the last few years (Cook, 2016). This has been associated with a range of factors including the outbreak of a serious abalone disease, illegal poaching and extreme climatic events that affected local populations (Cook, 2016; Mayfield, Mundy, Gorfine, Hart, & Worthington, 2012). Nonetheless, there have been no indications of local collapses, as documented for other countries (Mayfield et al., 2012). We found no evidence of genomewide diversity loss as might have been expected if populations were overexploited and experienced recent, severe and prolonged bottlenecks (Allendorf et al., 2012). In fact, diversity levels in greenlip abalone are comparable with red abalone from California (Wit & Palumbi, 2013), blacklip abalone from southeast Australia (Miller et al., 2016) and Roe's abalone from southwestern Australia (L. B. Beheregaray et al. unpublished data). On the other hand, demographic declines in other greenlip abalone subpopulations from the most heavily exploited zones in southeast Australia (Hart, Fabris, Brown, & Caputi, 2013; Shepherd & Rodda, 2000) could be attributed to reduced connectivity between them (Miller et al., 2014), as well as with the broader metapopulation identified here. It has been proposed that greenlip abalone metapopulation structure varies among biogeographic regions and that data on life history and recruitment dynamics are needed for guiding regional sustainable management (Miller et al., 2014). This agrees with seascape genetic studies spanning the entire southern Australian coast which show that metapopulation connectivity and recruitment success in broadcast spawning intertidal organisms vary along the coast in part due to differences in on-shelf oceanographic dynamics (Teske et al., 2015, 2017). The genomewide signal consistent with stepping-stone dispersal detected in our study emphasizes the importance of management policies aimed at maintaining demographically secure local subpopulations. This should be viewed as a key step in promoting broad-scale metapopulation connectivity and the long-term commercial viability of the greenlip abalone fishery.

5 | CONCLUSIONS

By integrating analyses of genomewide data with environmental mapping along a large longitudinal coastal region, we showed that

ecologically relevant adaptive divergence can be detected within a highly connected metapopulation of a broadcast spawner. Despite high connectivity promoted by the Leeuwin Current, a substantial number of genetic variants appeared to be under differential selective pressure along southern Australia's zonal coastal boundary. Environmental heterogeneity in oxygen concentrations and minimum temperatures influenced significantly the adaptive genetic variation along the coast. Several genetic variants under selection were annotated as genes whose functions putatively affect high temperature and/or low oxygen tolerance in other animals, including several abalone species. Therefore, these candidate genes are likely to influence the resilience of abalone populations in future environments. We also generated complementary information about spatial scales of metapopulation connectivity and adaptive divergence to be used during integrative management for fisheries and aquaculture. Given that the signal of environmentally mediated selection reported here appears to be polygenic, our list of candidate loci probably represents only a small fraction of the targets of selection present in the genomes of greenlip abalone populations. Additional studies covering a larger range of coastal selective environments and including information from whole-genome data sets, temporal population sampling and mechanistic experimental studies (McCairns, Smith, Sasaki, Bernatchez, & Beheregaray, 2016) are needed to better characterize and validate the genomic locations under selection, as well as to clarify the influence of recruitment dynamics and selective environments in shaping local adaptation of abalones.

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DATA ACCESSIBILITY

Reference sequences and SNP data set are available on Dryad: DRYAD entry https://doi.org/10.5061/dryad.1cf2p.

AUTHOR CONTRIBUTIONS

L.B.B. designed the study, with input from A.M.H. L.W.S.S. and A.M.H. provided the samples. J.S.C. generated and analysed the data with assistance from L.B.B. and N.A.R. J.S.C and L.B.B. led the writing of the manuscript. All authors contributed to interpretation of results and critically revised the manuscript.

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