




# Seascape genomics of coastal bottlenose dolphins along strong gradients of temperature and salinity

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## Abstract

Heterogeneous seascapes and strong environmental gradients in coastal waters are expected to influence adaptive divergence, particularly in species with large population sizes where selection is expected to be highly efficient. However, these influences might also extend to species characterized by strong social structure, natal philopatry and small home ranges. We implemented a seascape genomic study to test this hypothesis in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) distributed along the environmentally heterogeneous coast of southern Australia. The data sets included oceanographic and environmental variables thought to be good predictors of local adaptation in dolphins and 8081 filtered single nucleotide polymorphisms (SNPs) genotyped for individuals sampled from seven different bioregions. From a neutral perspective, population structure and connectivity of the dolphins were generally influenced by habitat type and social structuring. Genotype-environment association analysis identified 241 candidate adaptive loci and revealed that sea surface temperature and salinity gradients influenced adaptive divergence in these animals at both large- (1000 km) and fine-scales (<100 km). Enrichment analysis and annotation of candidate genes revealed functions related to sodium-activated ion transport, kidney development, adipogenesis and thermogenesis. The findings of spatial adaptive divergence and inferences of putative physiological adaptations challenge previous suggestions that marine megafauna is most likely to be affected by environmental and climatic changes via indirect, trophic effects. Our work contributes to conservation management of coastal bottlenose dolphins subjected to anthropogenic disturbance and to efforts of clarifying how seascape heterogeneity influences adaptive diversity and evolution in small cetaceans.

## KEYWORDS

adaptive resilience, cetacean, climate change, environmental heterogeneity, landscape genomics

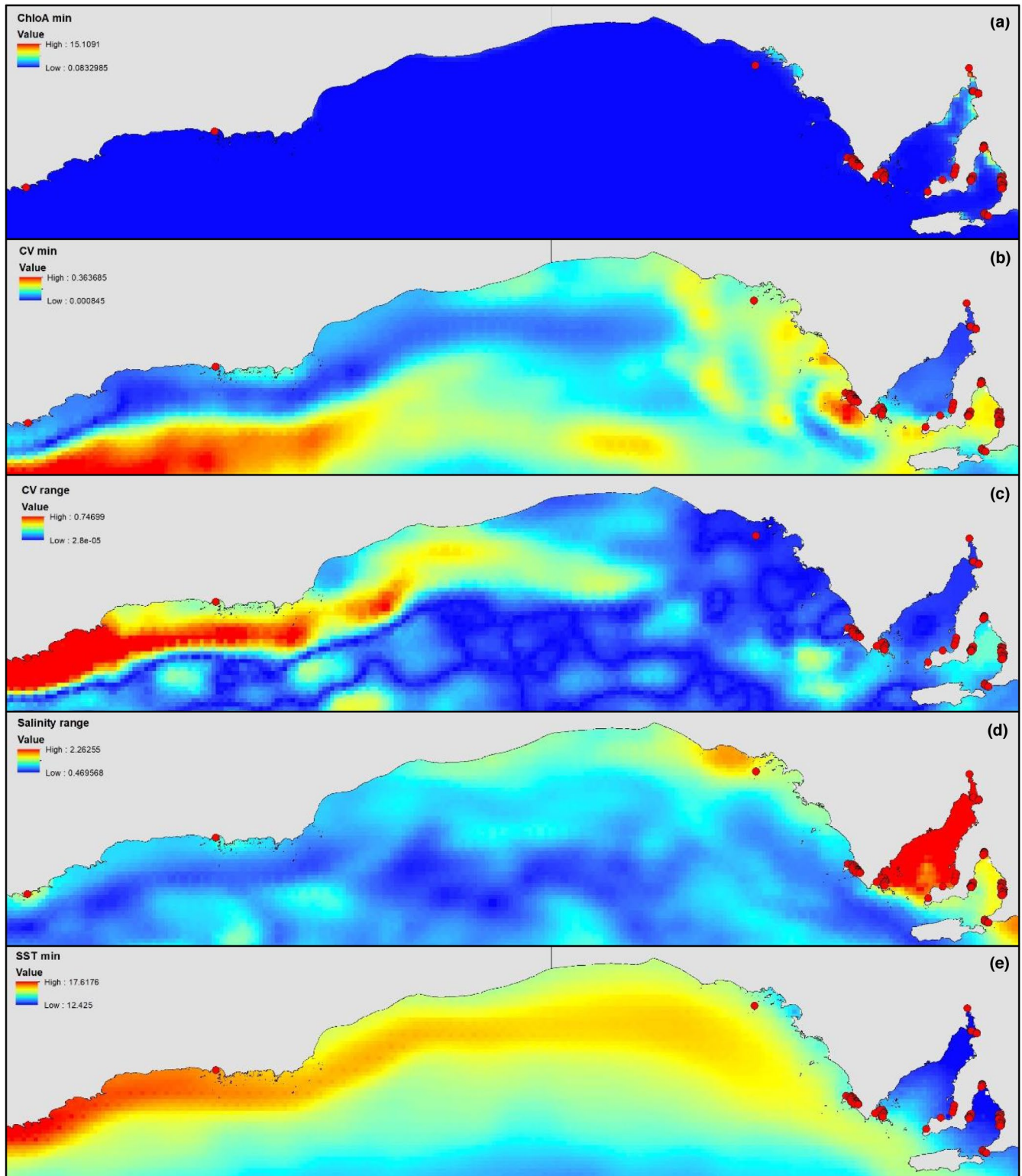
## 1 | INTRODUCTION

Adaptation due to divergent environmental conditions and colonisation of new habitats have been primary drivers of evolutionary change (Stroud & Losos, 2016; Wellborn & Langerhans, 2015). This can create subdivisions within a lineage, which is exacerbated when species exhibit strong social structure, natal philopatry and small home ranges (Storz, 1999). Such events of ecological divergence can take place in the presence of gene flow and not necessarily require physical isolation for the creation of new lineages (see Cooke et al., 2012). Neutral processes, such as mutation and genetic drift, are also important in genetic differentiation, particularly when population sizes are small (Willi et al., 2007). Both adaptive and neutral processes are therefore important factors to consider when investigating species and population-level differentiation.

In the marine environment, high gene flow and large-scale dispersal were once thought to be typical of marine populations. However, this paradigm is being questioned, with many species of teleosts and invertebrates showing finer-scale population structure than previously thought (e.g., Hoffman et al., 2012; Teske et al., 2015). Species of marine megafauna, including dolphins, sharks, pinnipeds and turtles, have also been documented to have population structure seemingly at odds with their highly mobile nature (e.g., Ahonen et al., 2016; Amaral et al., 2017; Bilgmann et al., 2007a; Dudgeon et al., 2009; Matsuzawa et al., 2016; Möller et al., 2011; Rodríguez-Zárate et al., 2018). These studies suggest that population structure may be influenced by environmental heterogeneity, indicating a more influential role for this phenomenon than previously thought. Landscape genomics presents an exciting framework to investigate the role of environmental heterogeneity in shaping population differentiation and adaptive divergence. This field of study investigates genome-wide neutral and adaptive variation of populations across heterogeneous landscapes to address novel or previously difficult questions, such as predicting adaptive capacity under environmental change (Grummer et al., 2019). While terrestrial landscape genomics has quickly become common place, the seascape equivalent has until now been largely focused on commercially important species of fish and invertebrates (e.g., Diopere et al., 2017; Sandoval-Castillo et al., 2018; but see Teske et al., 2019). The seascape genomics framework allows for empirical testing of relationships between particular environmental variables and genomic variation (i.e., genotype-environmental associations [GEAs]). Patterns of genomic variation based on neutral, compared to putatively adaptive loci, can also be untangled to reveal populations that may be strongly influenced by natural selection. The consideration of both adaptive and neutral information allows us to better understand processes shaping population differentiation and to evaluate the adaptive potential of species in response to environmental change (Funk et al., 2012; Grummer et al., 2019). This is key for the management of marine species as it ensures that different factors impacting on population structure and their relative vulnerability to disturbance are recognised and considered when developing marine park networks, catch limits, and other management strategies.

Many cetacean species have distributions spanning across vastly different habitats, making them an excellent group in which to study GEAs in the sea. Species and population-level differentiation in bottlenose dolphins (genus *Tursiops*) in particular, are thought to be influenced by environmental heterogeneity (Bilgmann et al., 2007b; Fruet et al., 2014b; Möller et al., 2007; Moura et al., 2013; Natoli et al., 2005). In southern Australia, coastal bottlenose dolphins belong to a lineage previously proposed to represent a separate species, *T. australis* (Charlton-Robb et al., 2011), but recent genomic data suggest it is most likely to represent a subspecies of *T. aduncus* (Moura et al., 2020; Pratt et al., in prep). Microsatellite and mitochondrial DNA studies have shown that this lineage is found only in coastal waters of southern Australia and is comprised of relatively small and highly structured populations (Bilgmann et al., 2007b; Charlton-Robb et al., 2015; Pratt et al., 2018). This narrow-endemic lineage of *Tursiops* is distributed over a highly heterogeneous seascape that spans coastal meso-scale bioregions with markedly steep gradients in sea surface temperature (SST), salinity and primary productivity, among others (Figures 1 and 2; Commonwealth of Australia, 2006; Day et al., 2008; Edyvane, 1999). More specifically, the environmental conditions in the western range of this lineage are influenced by the Leeuwin Current and the seascape is dominated by the Great Australian Bight, an approximately 1800 km stretch of open ocean with strong wave action and very few sheltered bays (Edyvane, 2000). Moving east along this longitudinal gradient, two large inverse estuaries in South Australia (Gulf St. Vincent and Spencer Gulf) provide protected habitat for many local marine species (Kämpf et al., 2010; Petrusevics, 1993). These gulfs are however, starkly different to one another, with an exceptionally strong gradient of hypersalinity in Spencer Gulf (Figure 1) and a more uniform habitat in Gulf St. Vincent (Commonwealth of Australia, 2006; O'Connell et al., 2016). Environmental heterogeneity across this region has been previously suggested to influence population genetic structure of the local dolphins (Bilgmann et al., 2007b; Pratt et al., 2018), similar to what has been proposed for coastal bottlenose dolphins in other parts of the world (Amaral et al., 2017; Möller, 2012; Natoli et al., 2005; Wiszniewski et al., 2010). Nonetheless, the general lack of genome-wide population data sets has so far prevented seascape genomics studies of adaptive divergence and GEA tests for coastal dolphins.

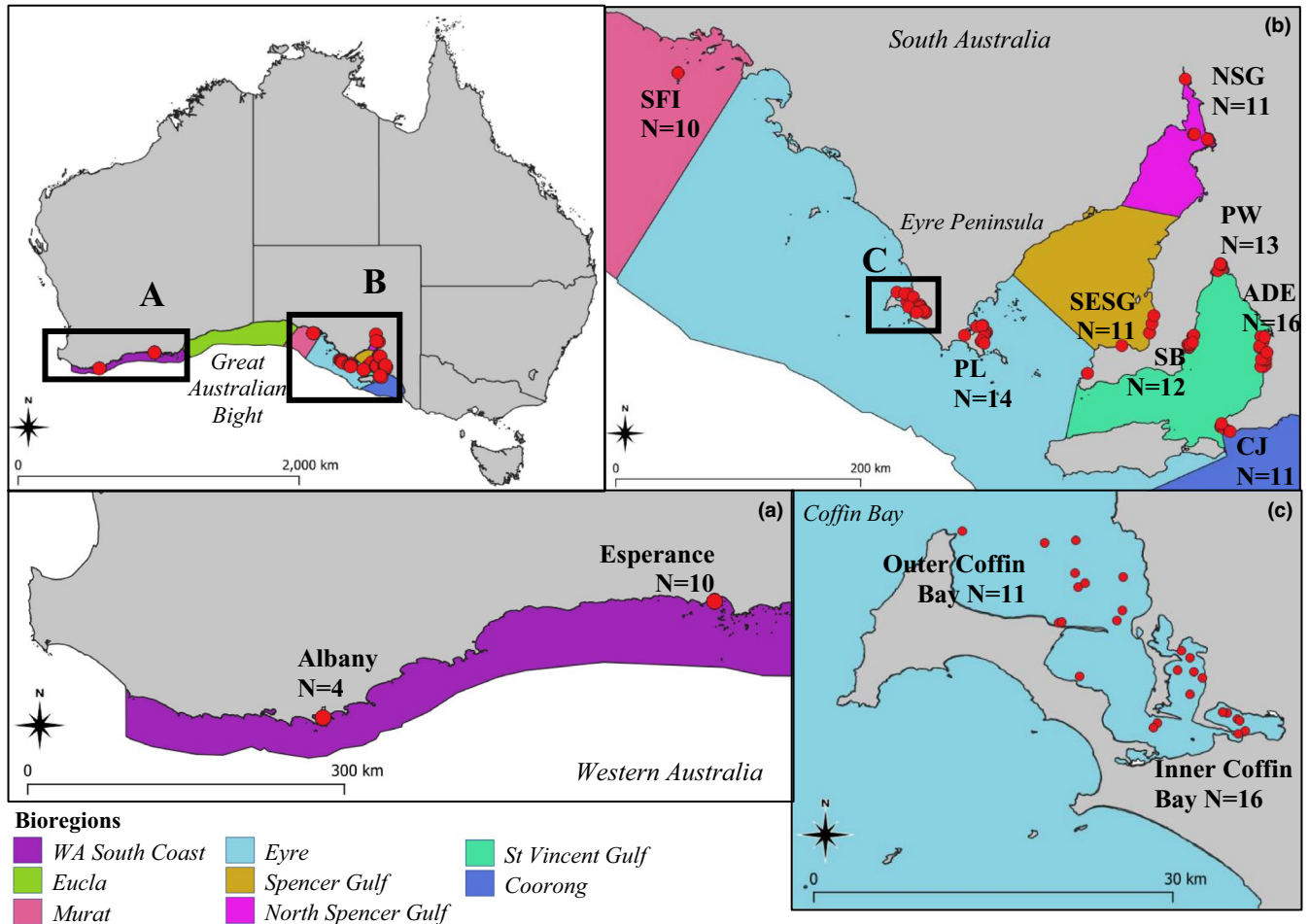
Coastal bottlenose dolphins are one of the best-studied cetacean species and marine predators worldwide (see Hoelzel et al., 1998; Möller & Beheregaray, 2001; Tezanos-Pinto et al., 2009 among others). They are often used around the globe as sentinels of coastal ecological processes and in Australia, being a protected species composed of fragmented populations, play a prominent role in the design, zoning and monitoring of marine parks (e.g., Passadore et al., 2018b). Dolphins in southern Australia are exposed to habitat disturbance and modification, tourism, pollution (Bossley et al., 2017), commercial and recreational fishing (Bilgmann et al., 2008), deadly viral outbreaks (Kemper et al., 2016) and a rapidly changing climate (Robbins et al., 2017). These types of impacts are not uncommon for many coastal dolphin lineages around the world, including *T. aduncus*



**FIGURE 1** Maps of spatial heterogeneity in the five environmental variables included in the final redundancy analysis (RDA) model run for genotype-environment association (GEA) analysis of southern Australian coastal bottlenose dolphins. (a) chlorophyll A concentration (chloA) minimum; (b) current velocity (CV) minimum; (c) CV range; (d) salinity range; and (e) sea surface temperature (SST) minimum. Further information on these environmental variables can be found in Table S2

(Reeves et al., 2003). As a result, the species is currently classified as near threatened by the International Union for the Conservation of Nature (IUCN). The vulnerability of the southern Australian-endemic

bottlenose dolphin lineage to population declines is exacerbated by its narrow endemism and limited knowledge about their ecology and demography. With high levels of anthropogenic impacts and ongoing



**FIGURE 2** Sampling sites of southern Australian coastal bottlenose dolphins (*Tursiops aduncus*) across the coastal meso-scale bioregions (Commonwealth of Australia, 2006). Note: SFI, St. Francis Island; PL, Port Lincoln; NSG, Northern Spencer Gulf; SESG, Southeast Spencer Gulf; SB, Stansbury; PW, Port Wakefield; ADE, Adelaide; CJ, Cape Jervis. N, sample size before filtering

climate change affecting their range, a crucial step is to identify their populations and establish how these dolphins have adapted to different environmental conditions. This information can then help inform management strategies by considering how these dolphins may respond to future changes in the environment.

Here, we capitalize on the environmentally heterogeneous seascape of southern Australia to carry out the first seascape genomics study of a dolphin species. We generated and integrated genome-wide information and environmental data sets to investigate population genomic structure and carry out GEA analyses. While we hypothesize that neutral population structure at regional and fine scales will be influenced by IBD and patterns of social structure and natal philopatry, we predict adaptive divergence to correlate with the strong environmental gradients and habitat discontinuities in the region. By focusing on oceanographic and environmental variables thought to be predictors of local adaptation in dolphins (Bilgmann et al., 2007b; Fruet et al., 2014b; Möller et al., 2007; Moura et al., 2013; Natoli et al., 2005), our work untangles adaptive from neutral genetic divergence along seascapes and identifies environmental factors and physiological processes that are putatively influencing adaptation in these animals. As such, the study contributes to

understanding how seascape heterogeneity influences the adaptive capacity to evolve under environmental change of these iconic and ecologically important marine organisms.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample collection

Biopsy samples were collected between 2004 and 2015 from 139 free-ranging southern Australian bottlenose dolphins (*T. aduncus*; hereafter referred to as “dolphins”). Sampling was carried out across 12 locations that span seven bioregions characterised by differing biological, oceanographic and environmental variables (see DEH, 2006; Figure 2). Biopsy samples consisted of skin and blubber and were obtained by a hand-held biopsy pole (Bilgmann et al., 2007a) or a remote biopsy gun (Krützen et al., 2002). To reduce the risk of resampling, individuals were observed for fresh biopsy wounds or scars and recognisable dorsal fin characteristics. No dependent calves were sampled. Samples were stored in either 90% ethanol or a salt-saturated solution of 20% dimethyl sulphoxide (DMSO) at



-80°C. Microsatellite data available from Pratt et al. (2018) were used to select individuals for genomic analysis with the aim of avoiding closely related individuals. This was based on a Queller and Goodnight's (1989) estimator in GENALEX (Peakall & Smouse, 2006, 2012), which was used to select one individual of each pair that had an estimated relatedness value of  $\geq 0.5$  (theoretical value for first-order relatives).

## 2.2 | Genomic methods

DNA was extracted from skin using a salting-out protocol modified from Sunnucks and Hales (1996). DNA quality and quantity were checked using a Qubit 2.0 fluorometer (Life Technologies), NanoDrop 1000 spectrophotometer (Thermo Scientific) and gel electrophoresis. The sex of each dolphin was available from Bilgmann et al. (2007b) and Pratt et al. (2018). Genomic libraries were prepared for ddRAD-seq using a modified protocol from Peterson et al. (2012) as detailed in Brauer et al. (2016). Pools of individually barcoded DNA samples were multiplexed across three libraries/lanes and sequenced at the South Australia Health and Medical Research Institute (SAHMRI) on an Illumina HiSeq2000 platform as single-end, 100 bp reads.

## 2.3 | Bioinformatics

Raw data files were demultiplexed and processed using DDOCENT v.2.2.19 (Puritz et al., 2014) as per Brauer et al. (2016). Custom BASH scripts were used to run VCFTOOLS to filter the resulting variant call file (VCF) as detailed in Table S1. Retained loci were then mapped against the *T. aduncus* genome, downloaded from the National Center for Biotechnology Information (NCBI) (GCA\_003227395.1 ASM322739v1). Only loci that aligned to this genome were retained for analysis.

## 2.4 | Neutral population diversity and structure

A combination of GEA analysis (see below) and three outlier detection methods (Methods S1) were used to identify candidate loci under selection. Candidates were removed from the full SNP data set to form the putatively neutral data set, hereafter referred to as the neutral data set. Expected and observed heterozygosities ( $H_E$  and  $H_O$ , respectively), the percentage of polymorphic loci (%PL) and Wright's inbreeding coefficient ( $F_{IS}$ ) were calculated for each sampling site in ARLEQUIN v.3.5.2.2. To investigate neutral population genomic structure, a model-based maximum-likelihood method was implemented in ADMIXTURE v.1.3.0 (Alexander et al., 2009). This was run with default settings, testing the statistical likelihood for between one and 16 populations in the data set ( $K$ ), which corresponds to the number of sampling locations plus minimum number of social groups as informed by previous studies (Diaz-Aguirre et al., 2019; Zanardo et al., 2018). The  $K$  value with the lowest cross

validation error was selected as the most likely number of populations. Principal component analysis (PCA) was run in R using the packages ADEGENET and FACTOMINER to visually inspect the clustering of individual samples (Francois et al., 2015; Jombart, 2008; Jombart & Ahmed, 2011). Informed by exploratory analyses, the PCA was rerun separately for sites to the east and west of Eyre Peninsula (separating gulf and open-coast sites) to enable detection of potential hierarchical structure.

Pairwise differentiation ( $F_{ST}$ ) between sampling locations was calculated in ARLEQUIN based on 10,000 permutations. Significance levels were corrected using the Benjamini and Yekutieli (2001) (B-Y) correction to reduce biases due to multiple testing (also see Narum, 2006), resulting in an alpha ( $\alpha$ ) of 0.0105. An analysis of molecular variance (AMOVA) was used to assess if patterns of population differentiation identified by the above methods are consistent with a hierarchical metapopulation structure as proposed by Pratt et al. (2018). This was done by combining localities in the following four groups: (1) Albany, Esperance, St. Francis Island and outer Coffin Bay, (2) inner Coffin Bay, (3) Spencer Gulf, and (4) Gulf St. Vincent). A Mantel test was run in the R package VEGAN (Oksanen, 2011) to test for IBD using linearised  $F_{ST}$  [ $F_{ST}/(1-F_{ST})$ ], with geographical distance measured in Google Maps as the closest along-shore distance between localities. This is the most likely path of travel for coastal bottlenose dolphins along the southern Australian coastline as suggested by aerial surveys (Bilgmann et al., 2018). BAYESASS3 (Wilson & Rannala, 2003) was used to estimate contemporary migration rates among five inferred populations (i.e., [1] Albany, Esperance and St. Francis Island, [2] outer Coffin Bay, [3] inner Coffin Bay, [4] Spencer Gulf and [5] Gulf St. Vincent) (see results). To investigate the potential role of Coffin Bay as a connection corridor between the Great Australian Bight and embayment-resident dolphins (Passadore et al., 2018a), outer Coffin Bay dolphins were considered as a separate population for the purposes of the BAYESASS3 analysis only. To reduce computational time, a subset of 5,000 SNPs were randomly selected from the neutral data set for this analysis. Allele frequency, inbreeding coefficient and migration rate mixing parameters were adjusted to optimise acceptance rates as suggested by Rannala (2007). Chain convergence was assessed by running the program three times with the same parameters of one hundred million iterations and ten million burnin steps, but a differing number of seeds for each run (500, 1000 and 1500). Congruence and convergence among the three runs was then checked in TRACER v.1.7.1 (Rambaut et al., 2018). Results of the three runs were very similar and as such the results of only the run with 1,000 seeds are shown here.

## 2.5 | Genotype-environment associations

A seascape genomics approach based on a GEA analysis was run on the full filtered SNP data set (Table S1) to identify SNPs under selection due to environmental heterogeneity across the study region. Nine oceanographic, environmental and topographic variables were selected based on their hypothesised influences on

genetic variation of bottlenose dolphins (Bilgmann et al., 2007b; Natoli et al., 2005; Querouil et al., 2010; Zanardo et al., 2018). These variables were SST, salinity, current velocity, chlorophyll-A concentration (chl<sub>a</sub>), primary productivity, bathymetry, sea-floor slope, seafloor rugosity and topographic relief. Averaged annual mean, maximum, minimum and range values were utilised for each variable, whenever possible, resulting in a total of 24 parameters. Data were downloaded from a variety of regional and global sources as detailed in Table S2 and were standardised prior to analysis.

A redundancy analysis (RDA) implemented in the R package VEGAN was used to assess GEAs (Oksanen, 2011). This multivariate approach was shown to outperform other GEA methods over a range of demographic scenarios, levels of selection, sampling designs and sample sizes, and in the presence of IBD (Forester et al., 2018; reviewed in Grummer et al., 2019). The RDA was conducted at the individual level, utilising the XY coordinates taken for each individual sample. To best represent the distance among samples, XY coordinates were transformed by first calculating pairwise seascape distances (i.e., not crossing land) in ArcGIS. These were then used by the R package MEMGENE (Galpern et al., 2014) to create Moran's eigenvector maps (MEMs). Combined, the MEM axes represent spatial genetic patterns at multiple geographic scales, which were then used as the "space" variable in the RDA (see details in Galpern et al., 2014). Although aerial surveys have suggested that these dolphins most probably travel following the coastline in southern Australia (Bilgmann et al., 2018), transformed seascape distances are likely to provide an adequate representation of spatial differences among these dolphins. Previous studies of Gulf St. Vincent and Coffin Bay dolphins revealed strong residency and small home ranges for these communities (Diaz-Aguirre et al., 2019; Passadore et al., 2018a; Zanardo et al., 2016), and a similar pattern is expected to be characteristic of the other dolphin communities represented here. The *forward.sel* function in R was used to perform a forward selection by permutation of residuals under a reduced model. This was done to select the ecological variables explaining a significant ( $p < .05$ ) portion of the genomic variation and to reduce collinearity among variables. Only the variable explaining the highest proportion of genomic variation was retained out of a highly correlated pair of variables. This was followed by variance inflation factor (VIF) analysis to eliminate any residual collinearity. Individual variables were removed until all those remaining had a conservative VIF of  $<3$ , as previously used in other cetacean studies (Christiansen et al., 2013; Goldbogen et al., 2015; O'Brien, 2007). The retained variables were included in the final RDA model and were also visually inspected for geographic heterogeneity in ArcGIS. Significance of the overall model and each individual explanatory variable were calculated through 1000 analysis of variance (ANOVA) permutations. Loci were identified as candidates of being under selection if they had a score greater than three standard deviations (SD) from the mean locus scores, which was calculated across all loci for each of the first three RDA axes (Forester et al., 2018). Correlation of the allele frequency of

these candidate SNPs with each of the retained environmental variables was calculated to establish which variable(s) each candidate was most associated with.

## 2.6 | Adaptive genetic diversity and structure

Loci identified by RDA as candidates were used to form the putatively adaptive data set (hereafter referred to as the adaptive data set). Molecular diversity measures were calculated in ARLEQUIN as per the neutral data set. ADMIXTURE was also run as per the neutral data set, and was used only for comparative purposes, since the assumption of Hardy-Weinberg equilibrium (HWE) is probably violated by the adaptive data set (Funk et al., 2012). Free from this assumption, a PCA was run for all localities, and splitting the sites to the east and west of Eyre Peninsula. Pairwise differentiation ( $F_{ST}$ ) between sampling locations was calculated for the adaptive data set in ARLEQUIN using the same parameters as for the neutral data set. An AMOVA was run to determine the significance of the putative population structure. A Mantel test was also used to test for IBD at the adaptive level (i.e., isolation by environment), using linearised  $F_{ST}$  and the same geographic distances calculated for the neutral data set.

## 2.7 | Functional enrichment analysis and annotation

Flanking sequences of 300 bp around each SNP were extracted from the *T. aduncus* genome (as used during filtering) for all 8081 SNPs, resulting in a 601 bp length fragment containing a SNP. BLASTN was then used to perform a basic local alignment search tool (BLAST) search from the nucleotide database available through NCBI (Altschul et al., 1990; Sayers et al., 2019) with an expectation (e) value of  $1E-6$ . BLAST2GO (Conesa et al., 2005) was then used to map and annotate all "blasted" loci, with an e-value of  $1E-3$ . Functional enrichment analysis was performed in BLAST2GO using a Fisher's exact test with an  $\alpha$  value of 0.05 to identify gene ontology (GO) terms that were over- or underrepresented in the adaptive data set compared to the reference (full) data set. To further investigate how environmental variables may be impacting differentiation, functional annotation was conducted for candidate loci that were moderately to highly correlated ( $<-0.4$  or  $>0.4$ ) with one or both of the top two variables explaining most of the variation in the genomic data set. A BLAST search against the higher quality *T. truncatus* genome (NIST Tur\_tru v1 reference annotation release 101) was run in the NCBI web BLASTN tool. Only candidates with an e-value of  $<1E-10$  and identity of  $>90\%$  were considered. Candidate genes were identified within 20 kilobases (kb) of the query sequence, as previously used in another study for this dolphin lineage (Batley et al., 2019). Gene functions were then investigated using the Swiss-Prot database in UniProtKB (Boutet et al., 2007; The UniProt Consortium, 2018).

### 3 | RESULTS

Sequencing of 139 dolphin samples produced 410 million raw reads (average of 2,720,842 reads per individual ( $SD \pm 2,631,273$ )). Eight individuals with >20% missing data were removed, resulting in a data set of 131 dolphins with an average of 2.9% missing data ( $SD \pm 3.2\%$ ). After a series of filtering steps to obtain the highest quality data (Table S1), 8,104 SNPs were retained. These loci were aligned to the *T. aduncus* genome and had a 99.65% alignment rate, leaving a final data set of 8,081 SNPs for analysis (Table S1).

#### 3.1 | Neutral population genomic diversity and structure

Outlier detection methods identified 264 candidate SNPs that were removed from the data set, leaving 7,817 putatively neutral SNPs for downstream analysis. Neutral genomic diversity of the dolphins was relatively high across the study region (Table S3). Patterns of neutral genomic diversity did not vary across major habitat types (e.g., embayment vs. open-coast dolphins). Inbreeding values were relatively low in all sampled sites, except for northern Spencer Gulf (NSG) (Table S3). ADMIXTURE estimated four genomic clusters in the data set, revealing strong neutral differentiation of Gulf St. Vincent dolphins from others along southern Australia, and a high level of admixture in outer Coffin Bay dolphins (Figures 3a1–4, see Figure S1 for cross validation values). When the sites to the east and west of Eyre Peninsula were analysed separately, west Eyre Peninsula sites displayed a clear differentiation between inner Coffin Bay and the four other sites (Figure S2A), and there was also strong separation between the two gulfs (Figure S3A). The AMOVA suggested the presence of hierarchical population structure, with the percentage explained by four populations (4.06%;  $p < .001$ ) slightly higher than among sampled sites within populations (3.44%;  $p < .001$ ) (Table S4A). Global  $F_{ST}$  across all samples was moderate at 0.072. Pairwise  $F_{ST}$  comparisons across sampling localities ranged from relatively low to high (Figure 4), with the highest differentiation between Western Australia sites and those from inner Coffin Bay eastwards. As expected, given the marked west to east degree of differentiation evident from the analyses above, there was also strong support for a pattern of IBD ( $r = .8682$ ,  $p < .0001$ ) (Figure 5). Overall, the results point to the presence of a hierarchical metapopulation across the study region that appears influenced by IBD. BAYESASS3 revealed varying and often asymmetric levels of contemporary migration that appear consistent with the inferred IBD and hierarchical metapopulation structure (Figure S4). For instance, contemporary gene flow was particularly high out of Gulf St. Vincent into the adjacent Spencer Gulf, as well as from inner Coffin Bay into the outer Coffin Bay population (Figure S4). Comparatively low proportions of non-migrants were inferred for outer Coffin Bay and Spencer Gulf populations, while the inner

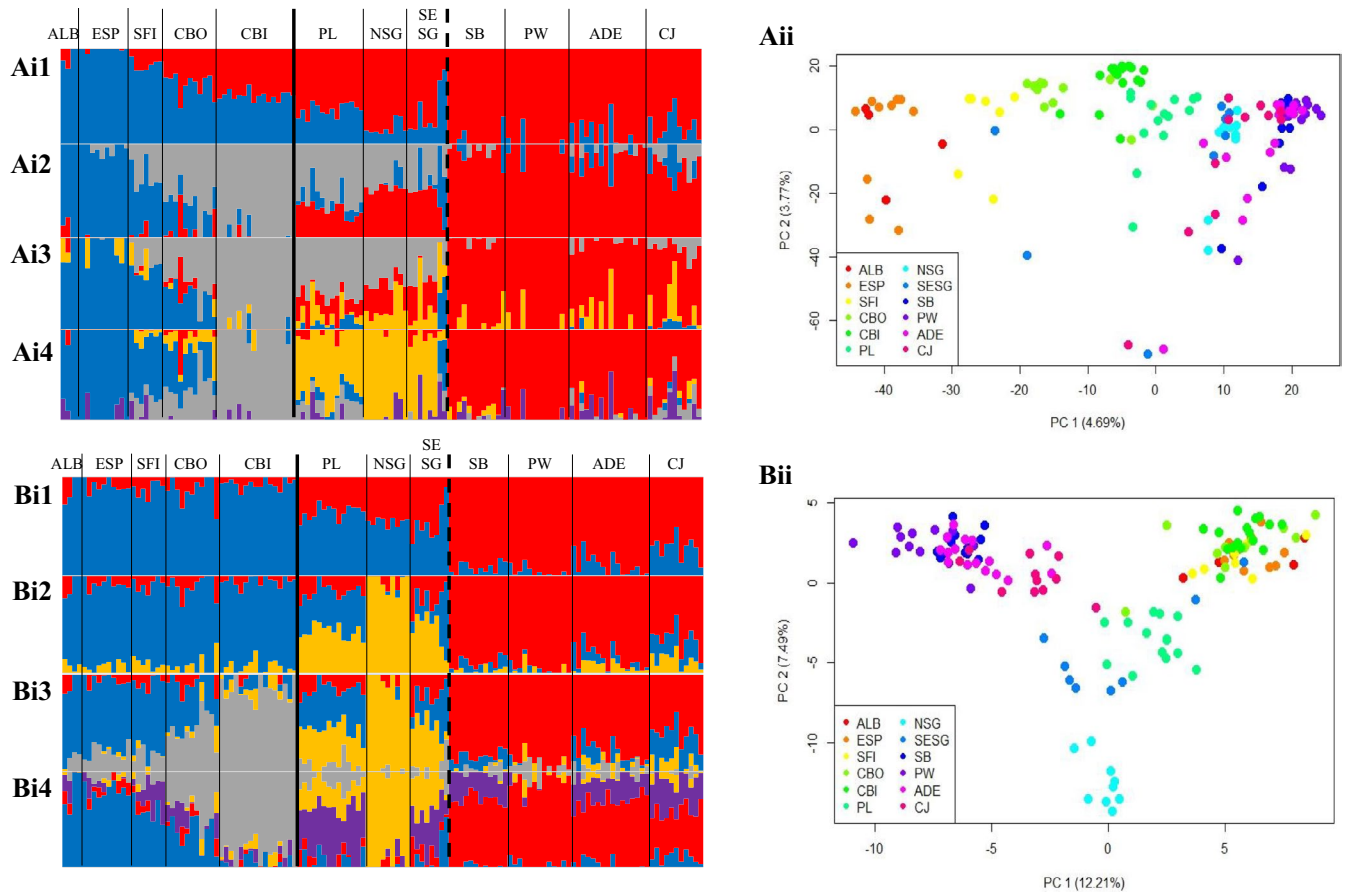
Coffin Bay embayment community had the highest proportion of nonmigrants (Figure S4).

#### 3.2 | Genotype-environment associations

A total of 24 environmental, topographic and oceanographic variables were used for the RDA (Table S2). Forward selection identified nine variables significantly associated ( $p < .05$ ) with genomic variation. After removing variables to reduce collinearity (e.g., minimum, maximum and mean salinity were excluded, Figure S5) the final five variables included in the RDA were minimum SST, minimum chl<sub>a</sub>, salinity range, and current velocity range and minimum. Strong variation in these five variables is evident across the region, as shown in the ArcGIS maps (Figure 1). The overall RDA model was significant ( $p = .001$ ), with 5.68% of the genomic variance in the data set explained by space and 9.30% explained by the retained environmental variables (Figure 6; Table S5). All five environmental variables were significant at  $p = .001$ , with minimum SST and salinity range explaining most of the variation. The RDA identified 241 candidate loci with scores  $\pm 3SD$  from the mean of at least one of the constrained RDA axes. On the first RDA axis (41.01% of the constrained variance), the inner and outer Coffin Bay dolphins separated from other localities based on an association with minimum SST and to some extent, minimum current velocity. Genomic divergence of Gulf St. Vincent dolphin communities, on the other hand, was associated with range in current velocity, minimum SST and minimum chl<sub>a</sub>. On the second axis (22.96% of the constrained variance), salinity range is associated with divergence of Spencer Gulf dolphins, particularly those in northern Spencer Gulf (Figure 6).

#### 3.3 | Adaptive population genetic diversity and structure

Molecular diversity of dolphins at the adaptive level (i.e., based on the 241 loci identified by the RDA) was high throughout the study region, with no clear association with habitat type or neutral population differentiation (Table S3). ADMIXTURE suggested four “adaptive populations”, with a very clear separation of Gulf St. Vincent dolphins (Stansbury, Pt. Wakefield and Adelaide) from all other sampling localities (Figure 3bi1–3, see Figure S1 for cross validation values). While the entire Spencer Gulf community also appears differentiated, NSG dolphins seem to be adaptively divergent from the southern Spencer Gulf (Port Lincoln and south-east Spencer Gulf) sites (Figure 3bi2–3, and Figure S3B). Southern Spencer Gulf dolphins appear to have stronger affinity to dolphins further west (Figure 3bi3); a finding also supported by PCA (Figure 3bii). Adaptive divergence of inner Coffin Bay was supported by the PCA run with the west Eyre Peninsula sites (Figure S2B). AMOVA showed that 12.03% of the variation ( $p < .001$ ) can be



**FIGURE 3** Population genomic structure of southern Australian coastal bottlenose dolphins (*Tursiops aduncus*) based on 131 individuals and (a) 7817 SNPs in the neutral data set and (b) 241 outlier SNPs in the adaptive data set. (i) Admixture plots whereby the thick black line represents the split between sites to the east and west of Eyre Peninsula and the dashed line marks the split between Spencer Gulf and Gulf St. Vincent. Population genomic structure is shown at (1)  $K = 2$  populations (neutral, high support; adaptive, low support); (2)  $K = 3$  (neutral, high support; adaptive, low support); (3)  $K = 4$  (neutral, most supported; adaptive, most supported); and (4)  $K = 5$  (neutral, high support; adaptive, high support); and (ii) Principal component analysis (PCA) plots. Sampling locations are ordered from west (left/top) to east (right/bottom). Note: ALB, Albany; ESP, Esperance; SFI, St. Francis Island; CBO, Coffin Bay Outer; CBI, Coffin Bay Inner; PL, Port Lincoln; NSG, Northern Spencer Gulf; SE SG, Southeast Spencer Gulf; SB, Stansbury; PW, Port Wakefield; ADE, Adelaide; CJ, Cape Jervis

explained by population-level differentiation, compared to 5.29% ( $p < .001$ ) among sampling localities within each population (Table S4B). Global  $F_{ST}$  at the adaptive level was substantially higher than for the neutral data set (Adaptive  $F_{ST} = 0.147$ , Neutral  $F_{ST} = 0.072$ ). Genetic and geographic distances were found to be moderately correlated ( $r = .3425$ ,  $p < .05$ ), but this was substantially less than that recorded for the neutral data set ( $r = .8682$ ) (Figure 5).

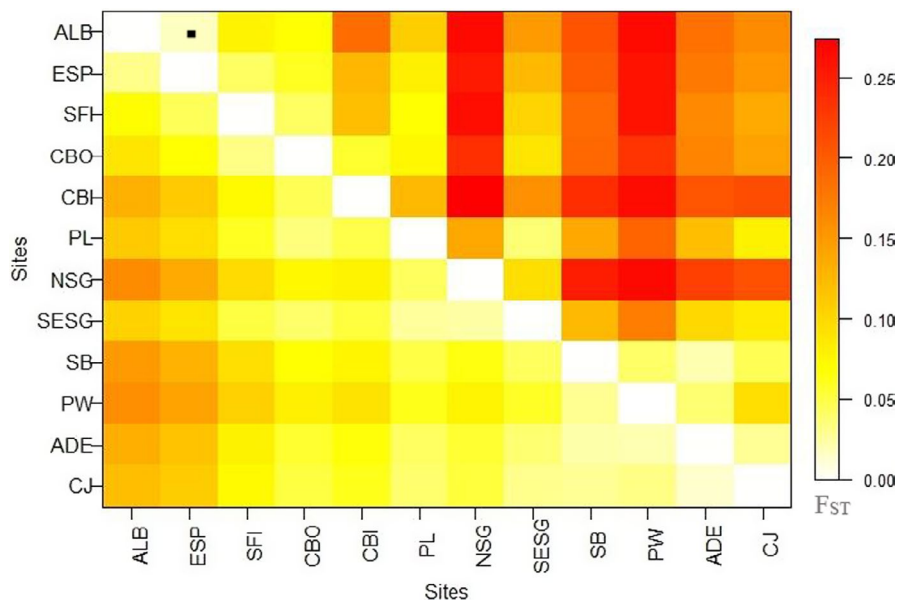
### 3.4 | Functional enrichment analysis and annotation

Of the 8,081 SNPs, 453 (5.6%) scored BLAST hits and were mapped and annotated, of which 3.3% (15) were GEA candidates. Functional enrichment analysis found 215 GO terms significantly ( $p < .05$ ) overrepresented in the putative adaptive data set compared to the full data set (no terms were underrepresented) (Table S6). Notable significantly over-enriched GO terms included temperature

homeostasis (GO:0001659), adaptive thermogenesis (GO:1990845), cellular response to carbohydrate stimulus (GO:0071322), positive regulation of muscle organ development (GO:0048636), ion gated channel activity (GO:0022839), ion transmembrane transport activity (GO:0015075), positive regulation of cell proliferation involved in kidney development (GO:1901724), as well as several other associated GO terms (significance values can be found in Table S6).

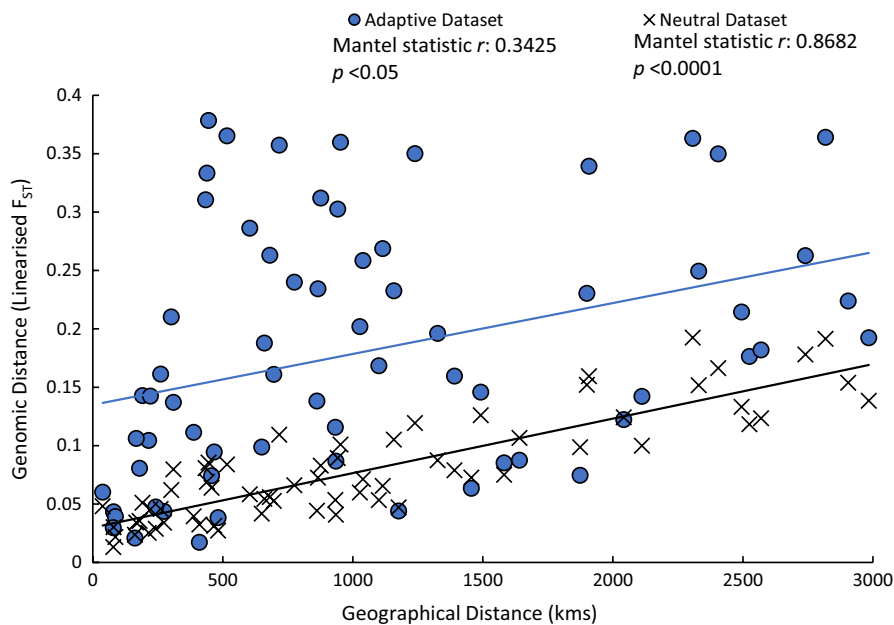
To further investigate individual gene functions, a total of 82 candidate loci that appeared moderately or highly correlated with salinity range and/or minimum SST (30 and 54, respectively, with two overlapping) were analysed with BLAST. Of these, 38 candidate genes were identified within 20KB of a candidate SNP (Table S7). Of particular interest was one candidate locus identified in an exonic region of the *CMKLR1* gene, which was negatively correlated with SST minimum. Allele frequency plotted across the southern Australian coast revealed an increase in homozygosity and frequency of the major allele closely corresponding to decreasing SST moving east (Figure 7a). The major allele





**FIGURE 4** Heat map of pairwise genomic differentiation ( $F_{ST}$ ) between sampling localities of southern Australian coastal bottlenose dolphins (*Tursiops aduncus*) based on 131 individuals as estimated by *Arlequin*.  $F_{ST}$  estimates based on 241 adaptive SNPs can be found in the top half of the matrix, while estimates based on the 7817 neutral SNPs are in the bottom half. The black square (■) denotes the one estimate that was nonsignificant at the B-Y corrected alpha value 0.0105 across all pairwise comparisons for both data sets. Note: ALB, Albany; ESP, Esperance; SFI, St. Francis Island; CBO, Coffin Bay Outer; CBI, Coffin Bay Inner; PL, Port Lincoln; NSG, Northern Spencer Gulf; SESG, Southeast Spencer Gulf, SB, Stansbury, PW, Port Wakefield, ADE, Adelaide and CJ, Cape Jervis

**FIGURE 5** Mantel test for isolation by distance (IBD) in southern Australian coastal bottlenose dolphins (*Tursiops aduncus*). Correlation between neutral (crosses) and adaptive (blue circles) genomic distance (linearised  $F_{ST}$ ) and along-shore geographical distance (km) among sampling localities is displayed

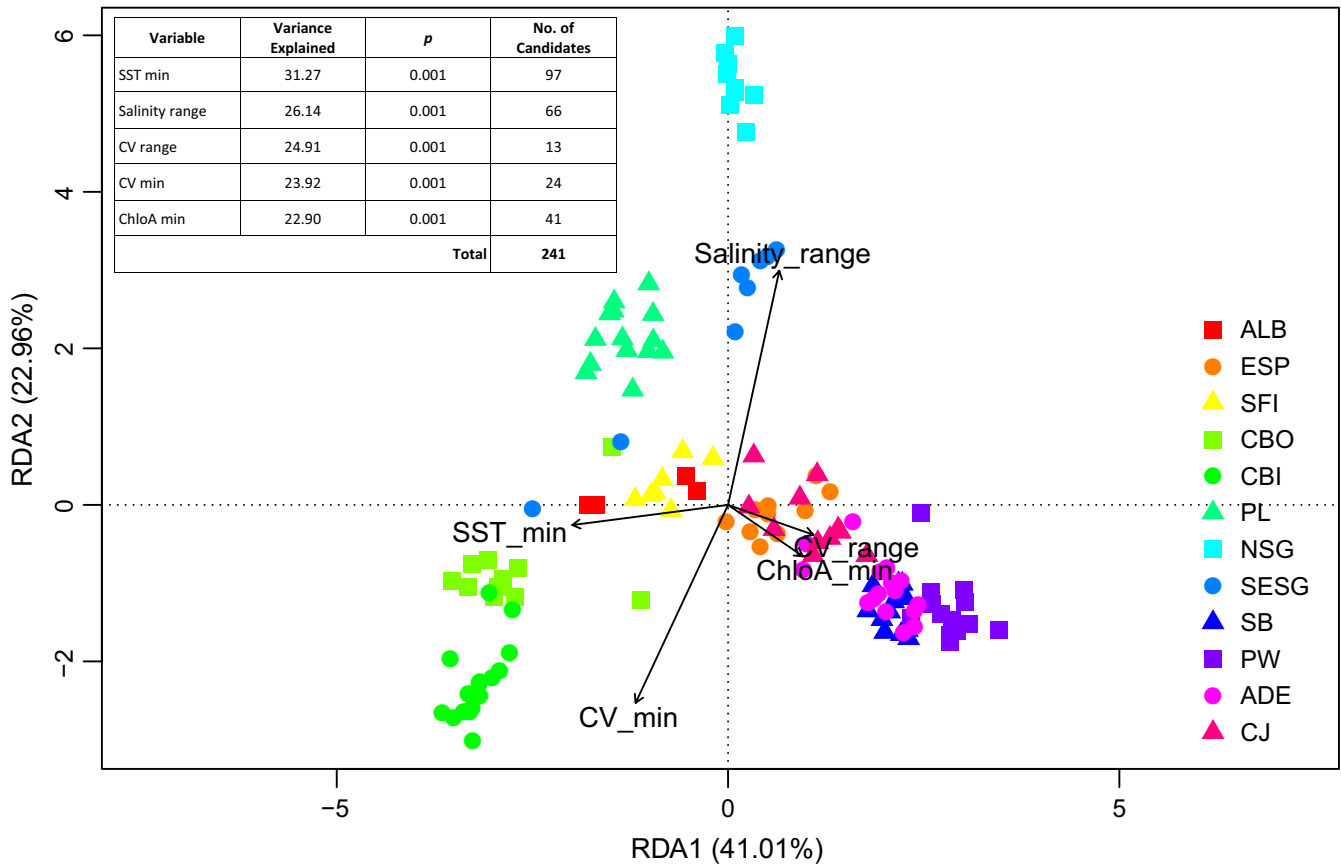


reaches near-fixation in Gulf St. Vincent, with 94% of inner-Gulf St. Vincent dolphins being homozygotes for this variant, compared to just 20% of dolphins to the west of Eyre Peninsula (Figure 7b). Several candidate loci that were highly correlated with salinity range were associated with genes involved in ion transport (Table S7). This included the genes *KCNT2* and *SLC22A18*, as well as an exonic region of *RYR2*. Variation in the allele frequencies of these genes shows a sharp increase in the frequency of the minor allele in Spencer Gulf, particularly NSG (Figure S6Ai, Bi and Ci). For all three genes, minor allele homozygotes are only present in Spencer

Gulf and are in particularly high frequency in NSG (Figure S6Aii, Bii and Cii).

## 4 | DISCUSSION

Anthropogenic climatic and environmental change is affecting all ecosystems on Earth, with a complex interplay of various changes which are occurring in marine ecosystems. This includes an increase in the frequency of climate extremes, such as marine heatwaves, and

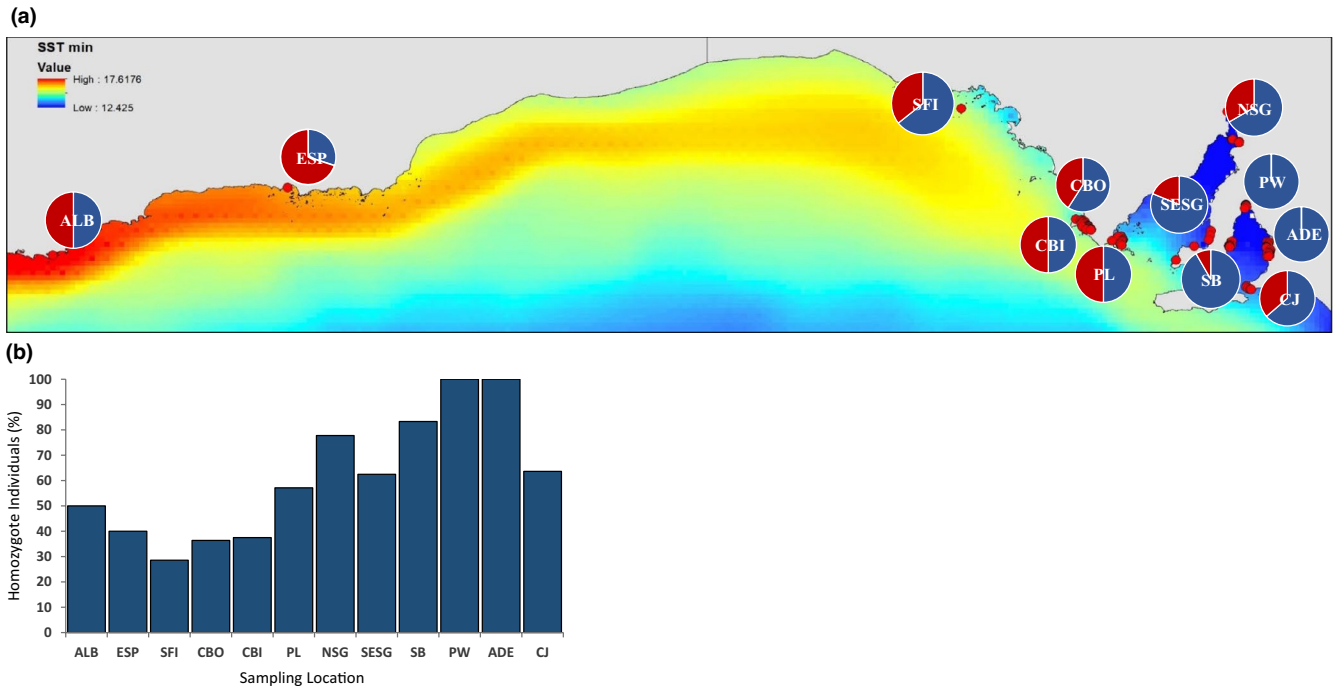


**FIGURE 6** Genotype-environment association redundancy analysis testing for the association between the five retained ecological variables and individual genomic differentiation in southern Australian coastal bottlenose dolphins (*Tursiops aduncus*), coloured by sampling locality. Overall variance in the genomic data set explained by the model was 186.10 ( $p = .001$ ) (1,702.40 residual variance), with 5.68% explained by space and 9.30% explained by the five ecological variables (see Table S5 for details). Table inset details the variance explained, significance ( $p$ ) and number of candidate loci most highly correlated with each of the five retained variables. Additional information of the environmental variables used are provided in Table S2 (SST, sea surface temperature; CV, current velocity, ChloA, chlorophyll A concentration; also note min, minimum). Sampling locations are ordered from west (top) to east (bottom) in the legend. Note: ALB, Albany; ESP, Esperance; SFI, St. Francis Island; CBO, Coffin Bay Outer; CBI, Coffin Bay Inner; PL, Port Lincoln; NSG, Northern Spencer Gulf; SESG, Southeast Spencer Gulf; SB, Stansbury; PW, Port Wakefield, ADE, Adelaide; CJ, Cape Jervis

an overall trend towards warmer, more acidic oceans (Poloczanska et al., 2013). While it is generally expected that upper trophic marine species may be mostly affected by such changes through indirect impacts on the food web and its habitats (e.g., Bakun et al., 2015; Scheffers et al., 2016; Sydeman et al., 2015), very little is known about how seascape and environmental heterogeneity influence their adaptive diversity. This is particularly true for coastal dolphins, a cosmopolitan group that features extensively in ecological and behavioural studies, as well as in conservation management frameworks and educational programmes about marine biodiversity. Here, we implemented a seascape genomics approach to clarify neutral and adaptive genetic divergence in open-coast and embayment populations of bottlenose dolphins from southern Australia. In agreement with previous microsatellite DNA and behavioural studies (Bilgmann et al., 2007b; Diaz-Aguirre et al., 2019; Pratt et al., 2018), we detected both broad and fine spatial population structure influenced by major habitat types and social structuring, respectively. Adaptive divergence on the other hand, was associated

with strong environmental gradients and changes in habitat types. Identified candidate adaptive genes that correlated with variation in salinity and/or temperature appear to be involved in cellular ion transport and adipogenesis. The evidence for spatial adaptive divergence, and probably physiological adaptations associated with environmental heterogeneity contrasts with previous suggestions that megafauna species are likely to be mainly affected by environmental and climatic change indirectly through impacts on their prey species (Bakun et al., 2015; Bilgmann et al., 2007b; Möller et al., 2011; Sydeman et al., 2015).

Extensive variation in genomic diversity among populations within species may be partially due to their demographic history (Ellegren & Galtier, 2016; Romiguier et al., 2014), including the effects of environmental and ecological disturbances (Bjørnstad & Grenfell, 2001; Ellegren & Galtier, 2016). In bottlenose dolphins, founder events, social structure and natal philopatry have been suggested as determinants of lower genetic variation in coastal compared to pelagic populations (Bayas-Rea et al., 2018; Louis



**FIGURE 7** Variation of candidate gene *CMKLR1* in southern Australian coastal bottlenose dolphins (*Tursiops aduncus*), showing (a) allele frequency change over the strong minimum sea surface temperature (SST) gradient across the seascape. Blue and red pie sections correspond to the frequency of occurrence at each sampling location of the major and minor alleles, respectively; and (b) the percentage of dolphins at each sampling locality found to be homozygotes at either allele. Note: ALB, Albany; ESP, Esperance; SFI, St. Francis Island; CBO, Coffin Bay Outer; CBI, Coffin Bay Inner; PL, Port Lincoln; NSG, Northern Spencer Gulf; SESG, Southeast Spencer Gulf; SB, Stansbury; PW, Port Wakefield; ADE, Adelaide; CJ, Cape Jervis

et al., 2014b; Möller, 2012; Möller et al., 2007). In our study however, overall differences in genomic diversity were not associated with broad-scale changes in habitat type (e.g., embayment/gulf vs. open-coast) for either neutral or adaptive data sets. Nonetheless, putative adaptive diversity was higher than neutral diversity at the spatial level of sampling locality. This suggests a potential influence of balancing selection within populations, whereby allelic diversity in genes affecting fitness is actively retained (Charlesworth, 2006; Hedrick, 2007). Alternatively, it could also be due to high natal philopatry and local adaptation caused by heterogeneous selection leading to an increase in genetic variation in the direction of selection (Assis et al., 2018).

#### 4.1 | BROADSCALE HABITAT TYPE INFLUENCES GENOME-WIDE POPULATION DIFFERENTIATION

Population differentiation in small cetaceans has often been linked to major changes in habitat type, which appears to influence local adaptation to specific prey types, which is then reinforced by social structure and philopatry (Hoelzel, 2009; Möller, 2012; Tezanos-Pinto et al., 2009). This is particularly common for bottlenose dolphin residing in embayments and other sheltered coastal habitats (Curry & Smith, 1997; Fruet et al., 2014a; Louis et al., 2014a; Möller et al., 2007). Accordingly, we detected clear neutral genetic differentiation among gulf, embayment and open-coast communities.

Broadly, a potential soft barrier to dispersal was identified off the Eyre Peninsula, consistent with previous findings based on micro-satellite data (Bilgmann et al., 2007b; Pratt et al., 2018). This may be related to a strong upwelling system south of Eyre Peninsula, which heavily influences fish distribution (Dimmlich et al., 2004; Kämpf et al., 2004). This influence could however, be somewhat confounded by the separation of the two gulfs from open-coast sites to the west (Figure 1b). Differences in environmental conditions either side of Eyre Peninsula appear to influence genetic differentiation in several marine organisms, such as common dolphins (*Delphinus delphis*; Bilgmann et al., 2014), Australian sea lions (*Neophoca cinerea*; Lowther & Goldsworthy, 2011), mulloway (*Argyrosomus japonicus*; Barnes et al., 2016), and perhaps clams (*Lasaea australis*; Li et al., 2013). The diversity of these examples points to the likely role of upwelling systems as a driver of population divergence of marine communities (also see Kelly & Palumbi, 2010).

A region that appeared highly relevant to connectivity of the dolphin metapopulation is the Great Australian Bight, including the waters off western Eyre Peninsula and the embayment of Coffin Bay (Figure 1a–c). This is part of the world's longest southern facing coastline, a region of global conservation significance due to high levels of biodiversity and endemism, and the presence of critical habitats and migratory pathways for many keystone species (Baghurst et al., 2017). Outer Coffin Bay dolphins appear to be facilitating a stepping-stone pattern of gene flow across ~1800 km between the highly philopatric inner Coffin Bay dolphin community (Passadore

et al., 2018a) and those from the open coast at the Great Australian Bight. This is supported by a strong signal of IBD and moderate to high contemporary migration to the west of Eyre Peninsula, as well as a lower proportion of nonmigrants. There is also lower density and encounter rates of dolphins in outer Coffin Bay than in the inner embayment based on photo-identification (Passadore et al., 2018a). The outer Coffin Bay dolphins therefore appear to exhibit a more transient lifestyle than is typical of inshore dolphins (i.e., Fruet et al., 2014a; Wiszniewski et al., 2010). This may be driven by instability in productivity and resources in outer Coffin Bay and this region's exposed coastline (Passadore et al., 2018b). Leeuwin and coastal currents probably support this phenomenon with seasonal changes in strength and direction, which may facilitate coastal movements in both directions across the Great Australian Bight (Feng et al., 2009). This finding challenges our current knowledge about range patterns and gene flow in coastal dolphins and demonstrates the broader impact of oceanographic and environmental features in shaping genomic differentiation across different trophic levels. Within Coffin Bay, spatial variation in fish assemblages (Whitmarsh et al., 2020) may be driving the dolphins' social division among the interconnected inner and outer bays (Díaz-Aguirre et al., 2019; Passadore et al., 2018a). This is probably linked to fine-scale resource and habitat specialisations.

Contemporary migration rates were relatively high between dolphins of the two gulfs (Gulf St. Vincent and Spencer Gulf), but negligible to low between gulf and outside gulf populations. In this region, the formation of summer/autumn thermal and saline fronts, and the presence of several islands at the entrances to both Gulf St. Vincent and Spencer Gulf, effectively sheltering the gulfs, and separating gulf and shelf waters (Harvey, 2006; O'Connell et al., 2016; Petrusevics, 1993). This oceanographic barrier was previously suggested to account for range differences between the coastal *T. aduncus* and the offshore-type *T. truncatus* (Gibbs & Kemper, 2014), as well as for genetic differentiation in *T. aduncus* (Bilgmann et al., 2007b; Pratt et al., 2018). Bottlenose dolphin gene flow could therefore be indirectly influenced through oceanographic impacts on prey populations and/or may be impacted by local adaptation to their respective gulf environments (discussed later). Gulf St. Vincent genomic differentiation to other dolphin populations may also partly reflect its colonisation history. This gulf was probably founded by a single bottlenose dolphin matriline (Pratt et al., 2018) as the gulf flooded relatively rapidly around 7000 years ago (Harvey, 2006), a hypothesis supported by low neutral genomic diversity in Gulf St. Vincent dolphins.

## 4.2 | Environmental gradients drive localized adaptive divergence in dolphins

Seascapes that encompass heterogeneous environments (e.g., distinct bioregions, habitat transitions) are expected to generate adaptive divergence in resident and philopatric populations due to the presence of ecologically divergent selection (Grummer et al., 2019;

Sandoval-Castillo & Beheregaray, 2020; Schluter, 2001; Teske et al., 2019). After controlling for spatial population structure (Forester et al., 2018), the GEA results pointed to adaptive divergence across bottlenose dolphin populations linked to strong gradients in temperature, salinity, oceanographic circulation and productivity. Although such genotype–environment links have been increasingly described for marine species (Grummer et al., 2019; Riginos et al., 2016; Sandoval-Castillo et al., 2018), this represents, to the best of our knowledge, the first such report for coastal dolphins. As discussed below, our GEA results and the functional inferences based on candidate genes suggest that seascape heterogeneity, and in particular differences between gulf/embayments and open coast, has influenced population adaptations both indirectly, as well as via putative physiological adaptations.

For Coffin Bay, GEA analysis indicated divergence between inner and outer embayment communities associated with minimum current velocity and salinity variation. Indeed, due to the shallow depths throughout inner Coffin Bay (mean = ~2.6 m, max = 5 m), the embayment presents strong variation in salinity (Kämpf & Ellis, 2015). Inner Coffin Bay has significantly reduced current velocity compared to outer, and connectivity with shelf waters is restricted (Kämpf & Ellis, 2015). Genetic differentiation associated with semi-enclosed estuaries is found in several small cetaceans, including common bottlenose dolphins (*T. truncatus*; Fruet et al., 2014a), Indo-Pacific finless porpoises (*Neophocaena phocaenoides*; Jia et al., 2014) and Franciscana dolphins (*Pontoporia blainvillei*; Costa-Urrutia et al., 2012). Estuaries are therefore likely to be an important habitat for the differentiation of cetaceans, potentially through the provision of niche space underutilised by other apex predators. This is also expected to apply to the two South Australian gulfs, which are both classified as large inverse estuaries (Commonwealth of Australia, 2006).

Three candidate genes, *KCNT2*, *SLC22A18* and *RYR2*, were identified to vary in correlation with the salinity gradient in southern Australia. For each of these genes, minor allele homozygotes were only present in Spencer Gulf, and were in particularly high frequency in the hypersaline conditions found in northern Spencer Gulf (Figure S6). *RYR2* has a crucial role in the regulation of heartbeat rhythm and is affected by intracellular sodium levels (Toischer et al., 2013). Increased omega-3 fatty acid consumption can have inhibitory effects on *RYR2*, reducing the rate of heart failure in humans (Ismail, 2005). The frequency of occurrence of several fish species with high levels of fatty acids (e.g., mackerel, tuna, herring and sardines) was observed to be much higher in the stomach contents of southern Spencer Gulf compared to the northern dolphins (Gibbs et al., 2011). The variation in an exonic region of this gene across the southern Australian coast may therefore, be a response to both the strong salinity gradient and prey choice differences in relation to heart function. Prey choice is typically thought to be associated with population genomic differentiation of cetaceans through its close relationship with sociality (e.g., Hoelzel et al., 2007). This finding however, suggests that there may also be a role for prey choice in the physiological adaptations of these animals.



Selection on *KCNT2* and *SLC22A18* on the other hand, may be associated with adaptation of a range of dolphin physiological systems in response to salinity and temperature variation. *KCNT2* codes for a sodium-dependent potassium channel typically activated by sodium elevation (Thomson et al., 2015). Low intracellular sodium concentration has been shown to deactivate these channels, making mice more susceptible to hypothermia and pain responses (Tomasello, 2017). *SLC22A18* however, controls transport of compounds in the kidney (Reece et al., 1998) and regulates fat accumulation, potentially relating to thermoregulation (Yamamoto et al., 2013). Adaptation of the renal system is further supported by significant over-enrichment of GO terms associated with kidney development and ion channel activity in the adaptive data set. Previous studies of macro- and microevolution in marine mammals, and cetaceans specifically, have documented members of the *KCN* and *SLC* gene families to be involved in adaptation to an aquatic lifestyle (Foote et al., 2015; Huelsmann et al., 2019; McGowen et al., 2012; Nery et al., 2013; Yim et al., 2014; Zhou et al., 2013, 2015, 2018). While physiological adaptation of cetaceans to salinity gradients is well documented at the macroevolutionary level, very few studies have investigated the genomic basis of this adaptation at a population level. Ruan et al. (2015) however, revealed that selection on genes associated with ion transport pathways and kidney development and functioning have been an important factor in the evolution of freshwater adaptation in finless porpoises (*N. asiaeorientalis* spp.). Selection on genes involved in these pathways have also been implicated in the development of freshwater tolerance in several teleost and invertebrate species (Czesny et al., 2012; Kozak et al., 2014; Lockwood & Somero, 2011). Genomic changes affecting ion transport and kidney development, among many others, are thus likely to be vital to osmoregulatory adaptation not only in cetaceans, but perhaps many marine species. With further temperature and salinity changes predicted under climate change, variations in homologs of these genes and gene families may therefore, be important in the adaptation of marine species into the future.

The GEA analysis identified several loci in the dolphin genome as candidates of being under selection in relation to the gradient in minimum SST. Subsequently, several GO terms related to thermogenesis, particularly cold-induced thermogenesis, were found to be significantly over-enriched. A candidate locus of particular interest was found in an exonic region of a homolog of the *CMKLR1* gene. The stark increase in the frequency of homozygotes and occurrence of the major allele moving east across southern Australia (Figure 7) suggests that directional selection could be acting on this gene, bringing the major allele closer to fixation in the cooler, eastern parts of the study area (see Hedrick, 2007). *CMKLR1* is closely associated with the process of adipogenesis (Audet et al., 2016; Ernst et al., 2012), influencing adipocyte (fat cell) differentiation from bone marrow precursor cells (Muruganandan et al., 2010). An inhibition of *CMKLR1* suppresses appetite and reduces weight gain and fat storage in mice (Ernst et al., 2012). Changes in the expression of this gene in the

hypothalamus of mice also affects individual core temperature and susceptibility to hypothermia (Audet et al., 2016). We hypothesise that *CMKLR1*, and potentially several other genes, create differing fat storage and thermogenic requirements in dolphins along the temperature gradient in southern Australia. Adipogenesis and volume of brown adipose tissue historically differ among human populations adapted to different climate zones (Sazzini et al., 2014). Genes associated with adipogenesis have also been found to differ between brown bears and polar bears, and between killer whale (*Orcinus orca*) ecotypes residing in regions characterised by starkly different temperature profiles (Foote et al., 2016; Liu et al., 2014). These studies not only linked the variation in adipogenesis-related genes to temperature, but also to differing diets. This has also been explored recently at the macroevolutionary scale, with lipid and glucose metabolism pathways undergoing major adaptations in the transition of cetaceans into an aquatic lifestyle and various diets (Derous et al., 2019; Nery et al., 2013). Accordingly, we found over-enrichment in GO terms associated with the cellular response to carbohydrate, monosaccharide, hexose and glucose stimuli. It could thus be speculated that in southern Australia, variation in *CMKLR1* and other genes in these dolphins may be associated with both SST and perhaps changes in prey choice across the seascape. Validating studies are needed to evaluate this further. It appears, however, that selection on genes related to adipogenesis is potentially a crucial factor in the adaptation of mammals to temperature differences and possibly associated changes in diet. While broad-scale temperature gradients across southern Australia may be driving differentiation of the gulfs from open-coast dolphin populations, salinity is also likely to be having a substantial impact for dolphins within the gulfs.

GEA analysis suggested that Gulf St. Vincent dolphins are differentiated from other dolphins based on an association with minimum SST, minimum chl<sub>a</sub> and variation in current velocity. These factors are largely influenced by the presence of Kangaroo Island in the Gulf St. Vincent mouth, affecting both the oceanography and geomorphology of this gulf (Edyvane, 2008). This could be having a direct influence on the local adaptation and genomic differentiation of these dolphins. Indeed, homozygosity in the temperature-associated candidate gene, *CMKLR1*, increases as one moves east across the study region, with 94% of the inner Gulf St. Vincent dolphins being homozygotes for the major allele. Cooler minimum temperatures in this region than elsewhere in southern Australia, particularly compared to shelf waters, are thus likely to be driving strong directional selection for this genotype in Gulf St. Vincent dolphins. This further highlights the potential importance of this gene and others associated with adipogenesis in adapting to temperature changes in marine species. While Gulf St. Vincent dolphins are collectively differentiated from others in the region, there is a high level of gene flow among dolphin communities within the gulf. This is in stark contrast to Spencer Gulf and may be facilitated by the relatively homogenous Gulf St. Vincent seascape, with only one bioregion recognised in this gulf (Figure 2, Commonwealth of Australia, 2006). This further demonstrates the

impact that habitat type and environmental heterogeneity may be having on bottlenose dolphin genomic differentiation.

### 4.3 | Conservation implications

Although more comprehensive than previous studies of GEA in bottlenose dolphins, the nature of a ddRAD data set means that the entire dolphin genome could not be investigated. Other genes, as well as complex gene interactions, are likely to be also influencing physiological processes and variation in phenotypic traits relevant to adaptation in coastal bottlenose dolphins in southern Australia. In addition, there may be other environmental and ecological variables of importance for which no data was available. Future research based on whole genomes, and which incorporate ecological interactions, is expected to improve our inferences about how seascape heterogeneity influences connectivity and adaptation in coastal dolphins, as well as the identification of gene regions and phenotypic traits involved in adaptive resilience. Despite these limitations, important ecological influences on bottlenose dolphin adaptation and neutral differentiation that are crucial to conservation management have been identified.

Southern Australian bottlenose dolphins are a divergent and narrow-endemic lineage that requires management targeted to its regional ecological conditions. Our study disclosed a hierarchical metapopulation of coastal bottlenose dolphins in southern Australia with differing levels of neutral and adaptive genetic divergence among populations. Previously, we could only define bottlenose dolphin management units based on neutral differentiation. We can now use information about adaptive diversity that provides a foundation to elucidate cryptic population differentiation relevant to the adaptive capacity of a lineage (e.g., Hoelzel et al., 2019). We recommend that any regional- and local-scale management strategies targeted at the dolphins directly, or at the network of marine protected areas, recognise (a) the strong genomic differentiation of Gulf St. Vincent bottlenose dolphins to others in southern Australia, (b) the genomic separation of the Spencer Gulf bottlenose dolphin population and the unique adaptive divergence of NSG dolphins, (c) the relative isolation and adaptive divergence of the small inner Coffin Bay bottlenose dolphin community, and (d) the importance of connectivity corridors along the Great Australian Bight for the wider coastal bottlenose dolphin metapopulation in southern Australia. Conservation and management plans also need to consider that there are at least four key environmental variables that are putatively influencing adaptive capacity of bottlenose dolphins in this region: temperature, salinity, primary productivity, and velocity of currents. Of these variables, increases in temperature and salinity due to climate change, were rated as the most significant threat to the long-term viability of marine mammals in the Spencer Gulf (Robbins et al., 2017). Climate change will probably result in strong selective pressure for traits involved in adaptation to changing conditions (Gienapp et al., 2008). It is therefore, important to safeguard the dolphins' standing genomic variation and connectivity across

southern Australia to allow movements and their capacity to respond to environmental change.

## 5 | BENEFIT-SHARING STATEMENT

Benefits generated: A research collaboration was developed with scientists around Australia providing genetic samples. All collaborators are included as coauthors, the results of research have been shared with the provider communities and the broader scientific community (see Data Availability Statement), and the research addresses a priority concern, in this case the conservation of bottlenose dolphins in coastal Southern Australia.

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### AUTHOR CONTRIBUTIONS

The study was conceived by Eleanor A.L. Pratt, Luciana M. Möller and Luciano B. Beheregaray. Samples were obtained by Eleanor A.L. Pratt, Luciana M. Möller, Kerstin Bilgmann, Nikki Zanardo, and Fernando Diaz-Aguirre. DNA extraction, ddRAD library preparation, bioinformatics and data analysis was done by Eleanor A.L. Pratt, with guidance from Chris Brauer, Jonathan Sandoval-Castillo, Luciana M. Möller and Luciano B. Beheregaray. The manuscript was written by Eleanor A.L. Pratt with revisions and drafting by Luciana M. Möller and Luciano B. Beheregaray, and additional comments from Kerstin Bilgmann, Nikki Zanardo, and Fernando Diaz-Aguirre.

### DATA AVAILABILITY STATEMENT

SNP genotypes and environmental data can be accessed on Dryad: 10.6084/m9.figshare.19100609.

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