Genomic signals of local adaptation in *Eleginops maclovinus* from Northern Chilean Patagonia

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Abstract

Understanding the evolutionary mechanisms that shape the adaptive divergence across spatially heterogeneous environments is a challenging task for evolutionary ecologists. The Chilean marine Patagonia is a complex ecosystem with diverse geomorphology and physical-chemical oceanographic conditions. There is limited research evaluating the interactions between selective forces and environmental conditions in this area. This study focuses on identifying the genomic signals of local adaptation of the endemic marine fish, *Eleginops maclovinus* from Chilean North Patagonia. To achieve this goal, we used an environmental marine database (temperature, salinity, oxygen, phosphate nitrate and silicate concentration) with collected from 1995 to 2018 and 11,961 SNPs obtained from 246 individuals from 10 sampling locations across this area. We identified putative adaptive loci using ten bioinformatic software tools, where five were based on population genetic differentiation (PGD) and five based on the genotype-environment association (GEA). We identified 392 adaptative loci using PGD and 2,164 associated with at least one of the six environmental variables analyzed using GEA. A total of 131 loci were shared between the PGD and GEA approaches, of which 37 were associated with genes involved in the growth, metabolism and homeostasis. Then, we evaluated the variation of adaptive loci with environmental variables using polygenic scores and found significant correlations with temperature, salinity, and oxygen, indicating polygenic selection along environmental gradients. This study highlights how polygenic selection drives local adaptation in *Eleginops maclovinus* and underscores the value of integrating genomic and environmental data for conservation in the Patagonian ecosystem.

***Keywords**:* Patagonian blennie, SNPs, candidate genes, seascape genomics, RAD-seq.

51 **1 Introduction**

52 The main aim of adaptive genomics is understanding the molecular basis of local
53 adaptation in species experiencing spatially heterogeneous environments. Currently,
54 detecting adaptative loci is possible using high-throughput sequencing technologies, which
55 allows thousands of single-nucleotide polymorphisms (SNPs) distributed across the genome
56 (Willette *et al.* 2014), to be genotyped in hundreds of individuals (Manel *et al.* 2016).

57 Restriction site-associated DNA sequencing (RAD-seq) method harness the high-
58 throughput sequencing technology to genotype individuals (Miller *et al.* 2007; Andrews *et*
59 *al.* 2016; McKinney *et al.* 2017a). Specifically, this method uses restriction enzymes that cut
60 at specific motifs throughout the genome to reduce their complexity (Altshuler *et al.* 2000;
61 Miller *et al.* 2007; Baird *et al.* 2008). The use of hundreds of thousands of RAD-seq markers
62 for exhibiting the signatures of selection is increasingly applied to marine organisms, offering
63 particular advantages for complex genomes in the absence of a reference sequence (Wang
64 *et al.* 2015; Benestan *et al.* 2016; Cure *et al.* 2017; Dalongeville *et al.* 2018; Hoey & Pinsky
65 2018; Li *et al.* 2019; Segovia *et al.* 2020; O'Leary *et al.* 2021).

66 For identifying adaptative loci, there are two predominant genome scan approaches: *(i)*
67 population genetic differentiation (PGD) and *(ii)* genotype-environment association (GEA).
68 The approach based on population genetic differentiation (PGD) identifies loci putatively
69 under selection by primarily comparing the genetic differentiation index (F_{ST}) and other
70 measures (e.g., inbreeding coefficient (F_{IS}) and locus-specific homozygosity excess) for each
71 locus against values predicted by a null model of neutral evolution. If natural selection favors
72 one allele at a particular locus in some populations, F_{ST} at that locus will be large compared

73 to loci in which among-population differences are the result of neutral demographic
74 processes (François *et al.* 2016). Contrarily, approach based on genotype-environment
75 association (GEA) search for associations between environmental variables and allele
76 frequencies across a set of populations spread over a geographic space (Rellstab *et al.* 2015;
77 Hoban *et al.* 2016). Alleles involved in local adaptation should occur at higher frequency
78 where they increase fitness and be at lower frequency where they decrease fitness. Therefore,
79 it may be possible to identify putative adaptative loci by scanning for alleles that show greater
80 than average genetic differentiation among populations using GEA approach (Lewontin &
81 Krakauer 1973; Beaumont 2005). These approaches are based on the premise that it is
82 possible to distinguish between the genetic signals left by adaptative and neutral processes.
83 However, algorithms, statistical models, and prior assumptions considerably differ and all of
84 them are prone to false positive associations (de Villemereuil *et al.* 2014; Rellstab *et al.* 2015;
85 de Villemereuil & Gaggiotti 2015; François *et al.* 2016). Combining PGD and GEA
86 outcomes may provide an efficient strategy to detect signatures of local adaptation while
87 reducing the number of false positives (de Villemereuil *et al.* 2014; Rellstab *et al.* 2015;
88 Gagnaire *et al.* 2015; François *et al.* 2016).

89 North Patagonian ecosystems in southern Chile are constituted by extensive and
90 interconnected system of fjords, channels, gulf, estuaries, and bays that are affected by
91 physical regimes that may strongly modulate biological productivity (Pantoja *et al.* 2011;
92 Iriarte *et al.* 2014). It extends approximately across 140,000 km^2 from latitude ~41.5ºS
93 (Reloncaví Fjord) to latitude ~46.5ºS (San Rafael Lagoon) (Rodrigo 2008).
94 Oceanographically, Chilean Northern Patagonia is considered a transitional marine system,
95 where the interaction between marine and fresh waters produces strong vertical and

horizontal gradients in physical-chemical variables (Pickard 1971; Dávila *et al.* 2002; Iriarte *et al.* 2014; Pérez-Santos *et al.* 2014; Schneider *et al.* 2014). These characteristics make Patagonia an excellent natural laboratory to investigate how this seascape heterogeneity results in genomics features. Thus, integrating genomic and environmental data provides a powerful approach for uncovering the processes leading to adaptative divergence in ecologically heterogeneous and connected marine ecosystems. So far, studies focused on seascape genomics in Northern Patagonia are limited (Araneda *et al.* 2016; Canales-Aguirre *et al.* 2016, 2018; Delgado *et al.* 2019; Segovia *et al.* 2020; Canales-Aguirre *et al.* 2022).

Eleginops maclovinus (Cuvier & Valenciennes 1830), is a notothenioid fish (Perciformes) of the monotypic family Eleginopidae (Pequeño 1989). This marine fish is endemic to the coastal temperate and sub-Antarctic waters of South America with a distribution range along the Atlantic and Pacific Patagonian coasts. In the Atlantic Ocean, it is distributed from Uruguay (~35°S) to the Beagle Channel (~54°S), including the Falkland/Malvinas Islands (Gosztonyi & Ae 1974; Eastman 1993), while in the Pacific Ocean, from the Beagle Channel to Valparaíso (~33°S) (Pequeño 1989). This species is a protandrous hermaphrodite with the highest fecundity (~796 eggs g^{-1}) (Brickle *et al.* 2005) among notothenioids (80-300 eggs g^{-1}) (Kock 1992; North 2001). It is euryhaline and can tolerate a broad range of salinity concentrations (5 and 45 PSU), however, extreme salinities can produce dramatic metabolic responses to fuel osmoregulatory tissues (Vargas-Chacoff *et al.* 2014, 2015a). Furthermore, juveniles of *E. maclovinus* have been described having a large eurythermal capacity, with tolerance between 4 and 20°C (Vanella *et al.* 2012; Oyarzún *et al.* 2018; Lattuca *et al.* 2018). All these studies described above have been conducted under controlled conditions, they are focused on one or two environmental variables, and do not

119 associate with genetic data. The latter leaves a gap on how loci under selection can shape the
120 adaptative capacity of *E. maclovinus* populations considering the complex environmental
121 variability in estuaries and fjords.

122 Studies about the geographic pattern of genetic diversity in *E. maclovinus* are limited
123 to three (Ceballos *et al.* 2012, 2016; Canales-Aguirre *et al.* 2022). A phylogeographic
124 research conducted by Ceballos et al. (2012) using mitochondrial DNA sequences for nine
125 locations did not find any evidence for the structuring of genetic variation into an Atlantic
126 and a Pacific group. Subsequently, using nine microsatellites it was revealed a low but
127 significant level of genetic differentiation between Pacific and Atlantic populations and
128 showed differences between locations in the Pacific coast (Ceballos *et al.* 2016). However,
129 the authors acknowledge that a single mitochondrial locus or nine microsatellites may not be
130 robust enough to allow them to recover significant subdivision among geographic regions.
131 Recently, Canales-Aguirre *et al.* (2022) used 12,050 SNPs in five locations distributed across
132 Northern Patagonia to identify neutral and adaptive population structure. However, the latter
133 only included five locations, used a public environmental database Bio-ORACLE
134 (Tyberghein *et al.* 2012; Assis *et al.* 2018) which is not accurate for fine-scale and fjords
135 areas like Patagonia, and only three approaches to identify adaptive loci. In addition, its focus
136 was to identify natural and adaptive loci for conservation and management purposes. To our
137 knowledge, this is the first study to identify the signal of local adaptation using ten different
138 bioinformatic approaches and six highly accurate environmental variables in populations of
139 *E. maclovinus* along Northern Patagonia.

140 In this study, we applied RAD-seq sequencing to generate SNPs genotyped in 246
141 individuals of *E. maclovinus* collected in North Patagonia. Here, we expanded the sampling

142 locations from Canales-Aguirre et al. (2022), increasing the number to ten locations to better
143 capture the environmental heterogeneity of their North Patagonian seascape. Our aim was to
144 detect SNPs putatively under selection using population genetic differentiation (PGD) and
145 genotype-environment association (GEA) approaches. We tested associations between SNPs
146 and six environmental variables (temperature, salinity concentration of oxygen, nitrate,
147 phosphate and silicate) obtained from oceanographic data recorded between 1995 to 2018 in
148 this area. We implemented a BLASTx search on putative adaptative SNPs identified by both
149 PGD and GEA approaches to investigate genes with molecular functions potentially involved
150 in local adaptation. Then, we evaluated the variation of adaptive loci using polygenic scores
151 where we found significant correlations and supporting polygenic selection in North
152 Patagonia.

153

2 Materials and Methods

2.1 Study area and environmental predictors

Chilean Patagonia is one of the most extended fjord regions in the world, located on the southeastern border of South America (Fig.1A), and composed by islands, peninsulas, fjords, and channels (Pantoja *et al.* 2011; Iriarte *et al.* 2014). This region has been affected by ice sheets calving into the ocean, retraction of the sea coast line and decrease in marine water temperatures since the Great Patagonian Glaciation (c. 1 Ma) during the Pleistocene (Rabassa *et al.* 2011). The Northern Patagonia is characterized by interactions between marine and fresh water, generating sharp vertical and horizontal salinity gradients (Dávila *et al.* 2002; Pérez-Santos *et al.* 2014; Schneider *et al.* 2014). The temperature shows a latitudinal gradient, which decreases southward until the Antarctic Polar Front (~55°S) (Iriarte *et al.* 2014). The oxygen concentration in the fjords is the result of horizontal advection of adjacent well-oxygenated Subantarctic Waters (5–6 mL/L) representing the major source of oxygen in the deep layers of the inner seas of Patagonia (Silva & Vargas 2014). In recent years, dissolved oxygen (DO) in the fjords of Chilean Patagonia has decreased by 1–2%, potentially driven by nutrient accumulation and rising temperatures (Linford *et al.* 2023a; b, 2024). The concentrations of inorganic nutrients show a strong seasonal signal, with high nitrate and orthophosphate concentrations during winter, and lower values during spring, presumably caused by a severe increase in primary productivity when light availability in near-surface waters increases (Iriarte *et al.* 2007; González *et al.* 2011; Torres *et al.* 2014; Jacob *et al.* 2014). The concentration of silicic acid shows latitudinal and longitudinal gradient due to surface water mixing between nitrate-rich but Silicic acid-

depleted oceanic subantarctic waters and Silicic acid-rich but nitrate depleted continental waters (Aracena *et al.* 2011; González *et al.* 2011; Torres *et al.* 2014).

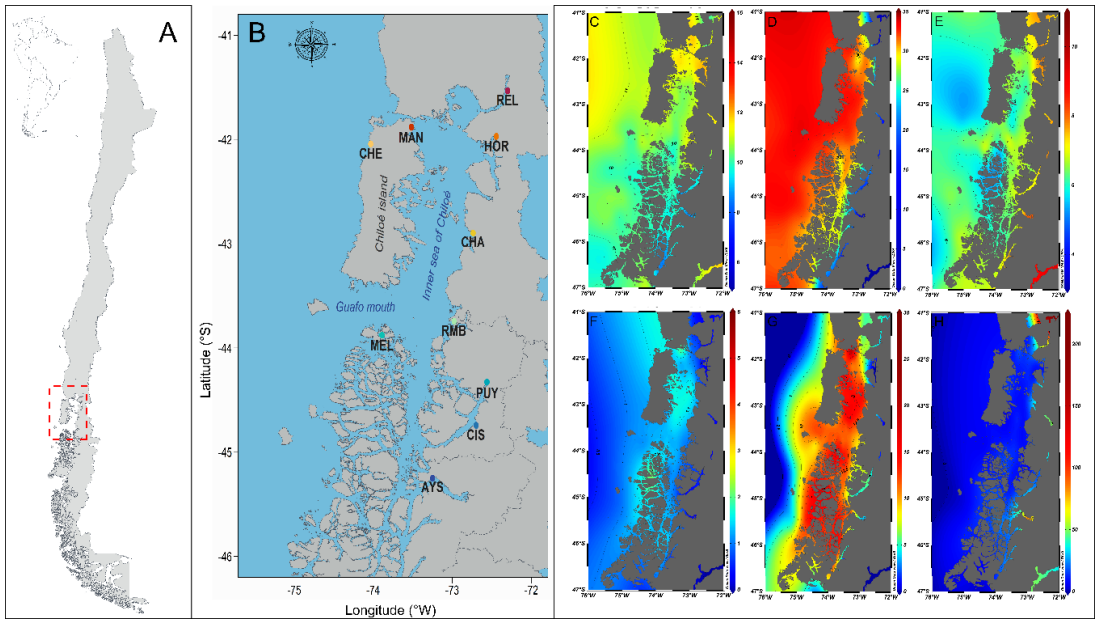

Figure 1 Geographic area and environmental variables in used.

Map of sampling locations in South America. B) Sampling site locations on North Patagonian fjord ecosystems in southern Chile REL: Reloncaví Estuary, MAN: Manao, HOR: Hornopirén, CHE: Chepu, CHA: Chaitén, RMB: Raúl Marín Balmaceda, MEL: Melinka Island, PUY: Puyuhuapi, CIS: Port Cisnes, AYS: Port Aysén: C) Mean surface temperature (ºC) D) Mean surface salinity (g/Kg); E) Mean surface concentration oxygen (µM); F) Mean surface nitrate (µM); G) Mean surface phosphate(µM); H) Mean surface silicate (µM).

We compiled an environmental database using data collected through the CIMAR Program of the National Oceanographic Committee of Chile (CONA), from CIMAR 1 (1995) to CIMAR 24 (2018). The database comprises 1,195 sampling stations located along the inner sea of Chiloé Island, estuaries, channels, and fjords from the Chilean Patagonia sampled in spring and winter (http://www.shoa.cl/n_cendhoc/productos/reporte_datos.php) (Fig. S1). At each sampling station, environmental variables were measured from the surface to the maximum possible depth. Considering that traditional databases lack high-resolution data near the coasts (e.g., Bio-Oracle, World Ocean Atlas, Aqua/MODIS), we highlight the use of information obtained by CIMAR as an effective alternative to detect the environmental heterogeneity of Northern Patagonia. Our database compiled contains six environmental predictors: conservative temperature[1] (ºC), absolute salinity[2] (g Kg^{-1}), oxygen (mL L^{-1}), nitrate (µM), phosphate (µM) and silicate (µM) concentrations. We used Ocean Data View v.5.4.0. (Schlitzer 2002) for data visualization. We estimated the mean, minimum, maximum and range of these environmental variables in each of the ten locations for a water column until 100 m depth using the entire period of the database (1995 – 2018) (Table S1). These latter, considering that *E. maclovinus* inhabits in coastal and estuarine areas at depths >40 m, and the spawning occurs in spring season (Brickle *et al.* 2005)

[1] Conservative temperature is a thermodynamic property of seawater. It is derived from the potential enthalpy and is recommended under the TEOS-10 standard (Thermodynamic Equation of Seawater-2010) as a replacement for potential temperature as it more accurately represents the heat content in the ocean.
[2] The Absolute Salinity is the mass fraction of dissolved non-H_2O material in a seawater sample at its temperature and pressure and is expressed in g Kg^{-1}

201 **2.2 Sampling and genotyping**

202 Between November 2018 and April 2019, we sampled 246 individuals of *E.*
203 *maclovinus* from ten locations throughout the North Patagonian fjord ecosystems in southern
204 Chile (Fig.1B). Five of the ten sampling locations included in this study were previously
205 analyzed by Canales-Aguirre *et al.* (2022). Sampling, DNA extraction, RAD-sequencing
206 library preparation, sequencing with Illumina technology and pipeline of bioinformatic
207 analyses were performed following procedures described by Canales-Aguirre *et al.* (2022).

208 Loci and samples were filtered iteratively by initially removing poor-quality data,
209 recalculating missing data proportions, and applying increasingly stringent thresholds,
210 following McKinney et al. (2020). The first part of the filtration process consisted in
211 excluding SNPs or individuals based on the following criteria *(i)* minor allele frequencies
212 (MAF) per sampling location, *(ii)* genotype rate per locus, *(iii)* genotype rate per sample.
213 Initially, filtration criterion was MAF ≤ 0.05, genotype rate threshold of 50% per locus and
214 sample. Subsequently, MAF≤ 0.1, genotype rate threshold of 90% per locus and sample. Due
215 to gene duplication events described to have affected the evolution of the notothenioid
216 genome (Xu *et al.* 2008; Chen *et al.* 2008, 2019), *HDplot* (McKinney *et al.* 2017b) was used
217 to identify paralogs, which were removed from further analysis (parameter flags: $H < 0.6$;
218 $|D| < 5$). We removed SNPs in Hardy-Weinberg disequilibrium (p-value < 0.05) if they
219 deviated in three or more locations to avoid potential issues such as inbreeding, population
220 stratification, and genotyping errors in the identified SNPs (Wigginton *et al.* 2005). For reads
221 with more than one SNP, we only kept those with the highest F_{ST}, to reduce the influence
222 from linked loci in the results. The resulting filtered (.vcf) file was converted into the

223 corresponding file formats for the subsequent analyses by using *PGDSpider* v. 2.1.1.5.
224 (Lischer & Excoffier 2012).

225 **2.3 Detecting putative loci under selection**

226 We employed ten different software programs to identify putative adaptive loci: five
227 based on population genetic differentiation (PGD) and five on genotype-environment
228 association (GEA). We combined multiple approaches to minimize false positive rates and
229 maximize our chances of detecting genomic signals of local adaptation (Rellstab *et al.* 2015;
230 François *et al.* 2016). For the PGD approach, we used *fsthet* v.1.0.1 (Flanagan & Jones 2017),
231 *BayeScan* v.2.1 (Foll & Gaggiotti 2008), *OutFLANK* v.0.2 (Whitlock & Lotterhos 2015),
232 *PCAdapt* v.4.3.3 (Luu *et al.* 2017; Privé *et al.* 2020), and *Arlequin* v.3.5.2.2 (Excoffier &
233 Lischer 2010) (Table 1). For the GEA approach, we used six environmental variables and
234 five software programs: *Latent Factors Mixed Model (LFMM)* (Frichot *et al.* 2013),
235 *BayeScEnv* (de Villemereuil & Gaggiotti 2015), *Redundancy Analysis (RDA).*(Hair *et al.*
236 1995; Zuur *et al.* 2010; Capblancq *et al.* 2018; Capblancq & Forester 2021)*, Moran Spectral*
237 *Outlier Detection (MSOD)* (Wagner *et al.* 2017), and *Samβada* (Stucki *et al.* 2017; Duruz *et*
238 *al.* 2019) (Table 2). In all programs, a 5% False Discovery Rate and respective p-values or
239 q-values were applied to detect putative adaptive loci. We employed *UpSet* diagrams (Lex
240 *et al.* 2014) to visually compare the results from the ten software programs, identifying loci
241 flagged as outliers by at least two programs as putative adaptive loci. For subsequent
242 analyses, loci were classified into two categories: putative adaptive loci (under directional
243 selection) and putative neutral loci, excluding loci under balancing selection.
244

245 **Table 1** Description and parameters used for software with the PGD approach.

Software	Description	Parameters	References
i) fsthet	*fsthet* identifies loci with extreme F_{ST} values by calculating smoothed quantiles for the F_{ST}-heterozygosity distribution, using its own empirical distribution instead of a null model due to changes in population structure.	We used a confidence level of 95% to detect F_{ST} outliers	(Flanagan & Jones 2017)
ii) *BayeScan*	*BayeScan* estimates the probability of selection for each locus using a Bayesian test that distinguishes between locus-specific selection effects (α) and population-specific demography effects (β), by measuring the discordance between global and population-specific allele frequencies.	We ran it with default parameters: 20 pilot runs of 5,000 iterations followed by 50,000 iterations with an additional burn-in of 50,000 and prior odd of 10.	(Foll & Gaggiotti 2008)
iii) *OutFLANK*	OutFLANK calculates a neutral distribution of F_{ST} values and assigns q-values to each locus to detect adaptive loci influenced by selection, without assuming a specific population's demographic history, reducing false positives.	We ran it with default parameters: LeftTrimFraction = 0.05, RightTrimFraction = 0.05, Hmin = 0.1.	(Whitlock & Lotterhos 2015)
iv) *PCAdapt*	*PCAdapt* performs a Principal Component Analysis (PCA) and computes the p-values for each locus to detect putative adaptative loci.	The most appropriate number of clusters K was chosen by testing K=1:10. We detected outliers using K=2 and p-values < 0.05 associated with Mahalanobis distances calculated with the q-value package.	(Dabney *et al.* 2010; Luu *et al.* 2017)
v) *Arlequin*	*Arlequin* calculates observed heterozygosity (H_O) to create a null distribution of F_{ST} values and associated p-values for each locus.	This was executed under a hierarchical island model with 20,000 simulations and 100 demes, which implements FDIST2 methodology.	(Excoffier & Lischer 2010)

246 **Table 2** Description and parameters used for software with the GEA approach**.**

Software	Description	Parameters	References
i) Latent factors mixed model (*LFMM*)	*LFMM* is a hierarchical Bayesian mixed model, which uses latent factors to correct population structure while fitting a linear regression between allele frequencies and environmental variables.	We estimated the number of ancestral populations K before the analysis testing from K 1 to 10 using the LEA v.3.2.0 R package and determined K from the cross-entropy criteria and Cattell's rule from the sNMF output. LFMM analyses were conducted separately for each environmental variable, with 10,000 iterations, and burning of 5,000 and five replicates.	(Cattell 1966; Devlin & Roeder 1999; Frichot *et al.* 2013, 2014; Frichot & François 2015)
ii) *BayeScEnv*	*BayeScEnv* detects putative adaptive loci by identifying loci that show large positive F_{ST} values (outside the neutral model F_{ST} distribution) that are significantly correlated with environmental variables.	We used 20 pilot runs, with 50,000 iterations each and an additional burn-in of 50,000 iterations for each of the six standardized environmental variables.	(de Villemereuil & Gaggiotti 2015).
iii) *Redundancy Analysis (RDA)*	*Redundancy Analysis (RDA)* is a multivariate ordination method to detect loci putatively under selection based on correlations with environmental variables.	We calculated the variance inflation factor (vif.cca function) to exclude variables with a value ≥ 5, and conducted linear regressions between allele frequencies and environmental variables at each locus. Using PCA, we produced ordination axes, identified putative adaptive loci (±3 SD from the mean), and evaluated the correlation using the Pearson correlation coefficient.	(Hair *et al.* 1995; Zuur *et al.* 2010; Rellstab *et al.* 2015; Forester *et al.* 2018)
iv) *Moran spectral outlier*	*MSOD* uses Moran eigenvector maps (MEM) to quantify the distribution of allele frequencies	We compared the power spectrum of each locus to the average power spectrum to identify outliers using z-scores and a cut-off of 0.05. Subsequently, we applied Moran	(Dray *et al.* 2006, 2021; Wagner & Dray 2015;

	detection (MSOD)	across a range of spatial scales represented by MEM spatial eigenvectors	spectral randomization (MSR) with 999 permutations using adespatial.	Wagner *et al.* 2017)
v)	*Samβada*	*Samβada* is a spatial approach that uses multiples univariate logistic regression models to identify locus-environment associations and at the same time measures spatial autocorrelation	We coded individuals for the presence or absence of each SNP allele and measured the association with environmental parameters across sites using multiple logistic regression. P-values from these regressions were calculated via a Wald Score Test and compared to a χ^2 distribution with one degree of freedom.	(Wald 1943; Stucki *et al.* 2017; Duruz *et al.* 2019)

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250 **2.4 Genetic diversity and population structure**

251 For the adaptative and neutral datasets, we determined, described and compared overall
252 and population-specific genetic diversity. The expected heterozygosity (H_E), observed
253 heterozygosity (H_O) and inbreeding coefficient (F_{IS}) were calculated for each locus and
254 locations using *Hierfstat* v 0.04.10 R package (Goudet 2005). We assessed the genetic
255 differentiation with pairwise F_{ST} (Weir & Cockerham 1984) comparisons between locations
256 using the R package *StAMPP* v.1.6.2. (Pembleton *et al.* 2013) and the statistical significances
257 were determined using 10,000 permutations.

258 We used two approaches to investigate the genetic structure: Bayesian clustering
259 program *Structure* v.2.3.4. (Pritchard *et al.* 2000) and discriminant analysis of principal
260 components (DAPC) (Jombart *et al.* 2010) using r package *adegenet* v.2.1.5. (Jombart 2008;
261 Jombart & Ahmed 2011). *Structure* is a Bayesian method, which uses a Markov chain Monte
262 Carlo (MCMC) algorithm based on the Gibbs sampler algorithm to identify the number of
263 putative genetic clusters. This method assumes Hardy-Weinberg equilibrium (HWE) and
264 linkage equilibrium among loci in sample population individuals (Pritchard *et al.* 2000). We
265 conducted the analysis using ten replicates per K value for K=1 to K=10, with 10,000 burn-
266 in steps and 100,000 MCMC-sampling steps. The runs were collected, sorted and merged
267 and functions in the R package *pophelper* (Francis 2017) to produce assignment barplots.
268 The most likely number of genetic clusters (K) was chosen using the ΔK method (Evanno *et*
269 *al.* 2005). The DAPC method identifies and describes clusters of genetically related
270 individuals from genomic datasets and allows the optimal visualization of between-

271 population differentiation in multivariate space (Jombart *et al.* 2010). First, we carried out a
272 principal component analysis (PCA), and used the principal components (PCs) thus produced
273 as synthetic variables for a discriminant analysis, as outlined in Jombart et al. (2010). The
274 optimal number of principle components was determined by cross validation (*xvalDapc*
275 function (Jombart *et al.* 2010)) to avoid overfitting.

276 We also conducted isolation by distance (IBD) analyses to test whether genetic distance
277 increased with geographic distance with adaptative and neutral data. We assessed the
278 correlation between the linearized pairwise F_{ST} matrix and the Euclidean (geographic)
279 distance matrix using a Mantel-test as implemented in the R-package *vegan* (Oksanen *et al.*
280 2016). Statistical significance was assessed with 999 permutations of the data. To test
281 whether the correlation between genetic and geographic distances is a result of a continuous
282 or distant patchy cline of genetic differentiation, local densities of distances to disentangle
283 both processes were plotted using 2-dimensional kernel density estimation (*kde2d* function)
284 of *MASS* R-package (Venables & Ripley 2013).

285 **2.5 Additive polygenic scores (APS)**

286 We assessed cumulative adaptive genetic variation by calculating additive polygenic
287 scores (APS) associated with each environmental variable (Gagnaire & Gaggiotti 2016;
288 Babin *et al.* 2017). For this analysis, we prioritized three biologically important
289 environmental variables: annual maximum temperature (°C), annual maximum salinity
290 (g/Kg), and annual minimum oxygen concentration (µM) (Holliday 1969; Nikinmaa & Rees
291 2005; Brian *et al.* 2008; Vanella *et al.* 2012; Vargas-Chacoff *et al.* 2014). We prioritized the

292 maximum values of temperature and salinity considering the effect that climate change has
293 on them. According to the Community Climate System Model 3.0 (CCSM3) of the National
294 Center for Atmospheric Research (NCAR), a 2.5°C increase in the temperature of Chilean
295 coastal areas is predicted by 2055 (Silva *et al.* 2016). Similarly, the Center for Oceanographic
296 Research in the Eastern South Pacific (COPAS) detected an increase of 0.25 in salinity in the
297 upper layers of the central-southern coast of Chile (Schneider *et al.* 2017)We also considered
298 the minimum oxygen concentration, as hypoxic conditions negatively impact fish fitness
299 (Richards 2009; Wu 2009). All these variables have an impact of the adaptive genetic
300 diversity of several species (Pauls *et al.* 2013; Bernatchez 2016)..

301 We assessed the relationship between each of the 131 putative adaptative loci and each
302 environmental variable. We used genotypes (0,1,2) to determine the score for each favored
303 allele; if the slope is positive, and (2,1,0) if negative (Babin *et al.* 2017). We calculated APS
304 by summing the scores of favored alleles associated with each environmental variable.
305 Finally, we evaluated the relationship between individual APS and environmental variables
306 using three models: linear, quadratic, and null, determining the best fit based on the lowest
307 Akaike Information Criterion (AIC) value.

308 **2.6 Loci annotation and Candidate Gene Identification**

309 Loci identified as putative adaptative loci (see results section 3.5) were functionally
310 annotated through Blast2GO v.6.0.3 (Conesa & Götz 2008). We compared the sequences
311 against bony fishes (taxa: 7898, Actinopterygii) to determine whether any putative adaptative
312 loci were associated with genes that may be related to environmental tolerances (e.g., heat

313 shock, immune system proteins, osmoregulation). We applied the BLASTx algorithm with a
314 cut-off of E-value $\leq 1x10^{-5}$, homology of sequences of more than 75% and other parameters
315 were used as default. Subsequent analysis only considered sequences with functional
316 annotation (blasted, mapped and annotated).
317

318 **3 Results**

319 **3.1 RAD sequencing and data filtering**

320 After quality control, 2,359,848 (511,190 RADtag) putative loci were obtained for Stacks
321 pipeline. Using the iterative filtering process (MAF and missing data), we identified a total
322 of 2,328,560 (98.67%) putative singletons and 386 putative paralog loci; the latter were
323 removed (1.24%). Further filtering by HWE discarded 13,215 loci (42.96%) and one SNP
324 per tag (the highest F_{ST}) discarded 5,582 SNPs (31.82%). A total of 11,961 SNPs (5.01%)
325 were retained, from 202 individuals across the 10 sampling locations (Table 3).

326 **Table 3** Description of steps used to filter SNPs

Filtering steps	Sample size	Number of loci
SNP after STACKS	246	2,359,848
[1]MAF >= 0.05	246	1,494,046
[1]Genotyped by locus (50%)	246	167,752
[1]Genotyped by sample (50%)	236	167,752
[1]Genotyped by locus (90%)	236	54,694
[1]Genotyped by sample (90%)	202	54,694
[1]MAF >= 0.1	202	31,144
Singletons (HDPlot H=0.6; D=5)	202	30,758
Hardy Weinberg equilibrium in 100% locations	202	17,543
one SNP by tag	202	11,961

327 [1] Steps included in the iterative filtering process

328 **3.2 Identification of loci putatively under selection**

329 From population genetic differentiation (PGD) and genotype-environment association
330 (GEA) we identified different putative adaptative loci. Out of the 11,961 loci, we identified
331 392 loci under positive selection by PGD, and 2,164 loci using the GEA approach. A total of
332 131 loci were identified as shared between the PGD and GEA approaches (Figure 2).

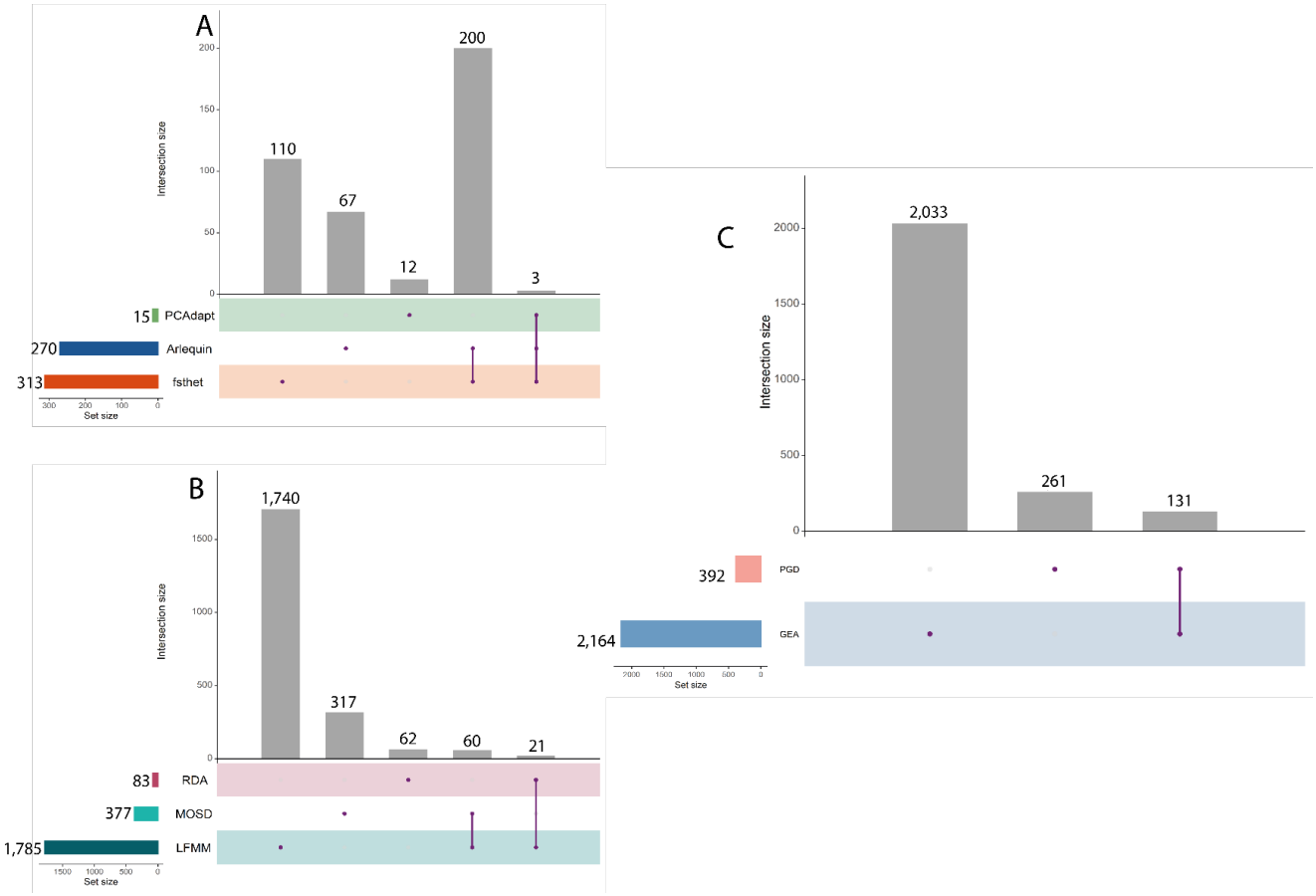


Figure 2 UpSet diagram: matrix layout for all intersections of software used. A) Loci detected by software based on Population Genetic Differentiation (PGD), B) Loci detected by software based on Environment Genotype-Association and C) Loci shared between PGD and GEA. The set size (horizontal bars) indicates the total number of loci detected by that software. The intersection size (vertical bars) indicates the number of loci per set intersection, the purple dots represent the set of unique loci detected only by that software and no other one, while the purple dots connected by a line represent loci shared by two, three, or more software. The *OutFLANK, BayeScan, BayeScEnv,* and *Samβada* software are excluded since they did not identify any adaptative loci**.**

342 Within PGD approaches, *fsthet* detected 334 SNPs (2.79%) under balancing selection and
343 313 SNPs (2.62%) under divergent selection (Figure S2). *Arlequin* identified 270 SNPs under
344 divergent selection (2.26%) (Figure S3). *PCAdapt* detected 15 SNPs (0.13%) under divergent
345 selection (Figure S4). *BayeScan* and *OutFLANK* found no outlier. For GEA approaches,
346 *LFMM* identified 1,785 loci under divergent selection, with the highest number of
347 associations to nitrate concentration (n = 684 loci) (Figure S5). *MSOD* detected 377 loci,
348 with the highest number of associations to temperature (n = 377 loci) (Figure S6). Finally,
349 *RDA* detected 83 loci under divergent selection, with the highest number of associations to
350 silicate (33 loci) (Figure S7). *Samβada* and *BayeScEnv* did not detect any correlation with
351 environmental variables. Overall, across GEA approaches, the nitrate concentration (n =
352 844), temperature (n = 748), and salinity (n = 703) were the environmental variables with
353 which most of the loci were correlated (Figure 3). Of the total of 2,164 that correlated with
354 each of the six environmental variables, 783 of the loci were significantly associated with
355 more than one environmental variable.

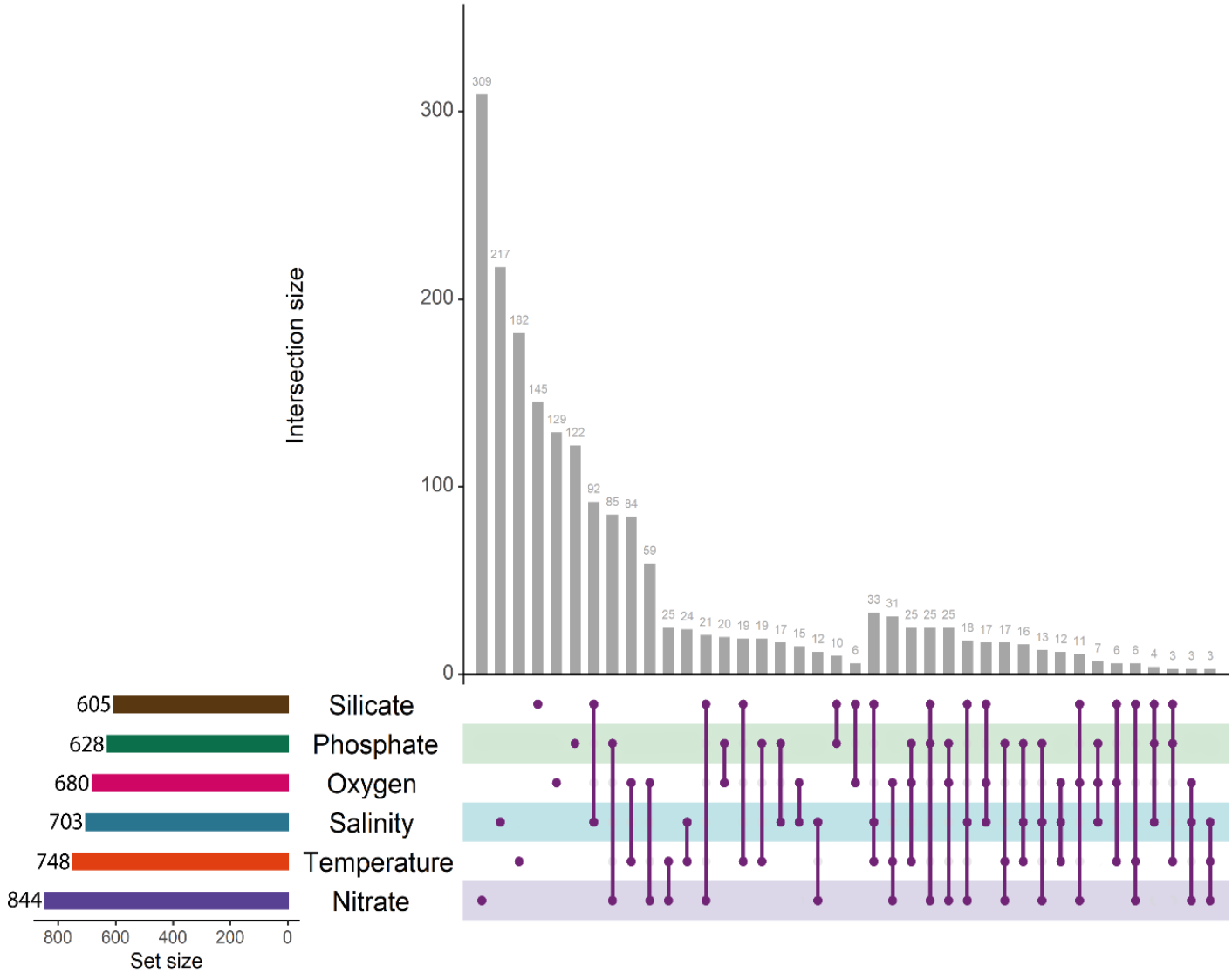


Figure 3 UpSet diagram: matrix layout for all intersections of environmental variables. The set size (horizontal bars) indicates the total number of loci correlated with that environmental variable. The intersection size (vertical bars) indicates the number of loci per set intersection, the purple dots represent the set of unique loci correlated only with that

361 environmental variable and no other one, while the purple dots connected by a line represent

362 loci shared by two, three, or more variables.

363 **3.3 Assessment of genetic diversity and population structure**

364 Summary statistics of genetic diversity revealed similar values for each location within
365 each dataset (Figure S8). Most locations showed lower values of observed heterozygosity
366 (H_O) than expected heterozygosity (H_E), and positive values of inbreeding coefficient $F_{IS.}$ For
367 the adaptative loci dataset, H_O 0.3364 ± 0.0078 ($C.I._{95\%}$), H_E 0.3551 ± 0,0068($C.I._{95\%}$), and
368 F_{IS} 0.0528 ± 0.0120 ($C.I._{95\%}$). For the neutral dataset, H_O 0.3414 ± 0.0074 ($C.I._{95\%}$), H_E 0.3485
369 ± 0,0063 ($C.I._{95\%}$), and F_{IS} 0.0206 ± 0.0117 ($C.I._{95\%}$).

370 Pairwise-F_{ST} comparisons showed higher levels of genetic differentiation for adaptive
371 loci (global F_{ST} = 0.0419 ± 0.0042 $CI_{95\%}$), than neutral loci (global F_{ST} = 0.0004 ± 0.0004
372 $CI_{95\%}$). For adaptative loci, all pairwise differentiation values were highly significant (p-value
373 ≤ 0.0004). While in neutral loci, 26 of 45 pairwise differentiation values showed significant
374 p-values (Table 4).

Table 4 Pairwise F_{ST} (below diagonal) and p-value (above diagonal). Values between sampling site locations from North Patagonian fjord ecosystems in southern Chile using A) adaptative loci and B) neutral loci. Statistical significance was considered below 0.05 p-value.

A)	REL	MAN	HOR	CHE	CHA	RMB	MEL	PUY	CIS	AYS
REL	-	0	0	0	0	0	0	0	0	0
MAN	0.0672	-	0.0004	0	0	0	0	0	0	0
HOR	0.0495	0.0126	-	0	0	0	0	0	0.0004	0
CHE	0.0653	0.0443	0.0493	-	0	0	0	0	0	0
CHA	0.0665	0.0502	0.0503	0.0531	-	0	0	0	0	0
RMB	0.044	0.0302	0.0227	0.0502	0.0502	-	0	0	0	0
MEL	0.0755	0.0343	0.0296	0.0521	0.0539	0.0313	-	0	0	0
PUY	0.0467	0.0403	0.0266	0.0546	0.0408	0.0286	0.033	-	0	0
CIS	0.0592	0.0338	0.0210	0.0546	0.0435	0.0248	0.0355	0.0234	-	0
AYS	0.0472	0.0442	0.0199	0.0553	0.0456	0.0269	0.0432	0.0263	0.0292	-

B)	REL	MAN	HOR	CHE	CHA	RMB	MEL	PUY	CIS	AYS
REL	-	0	0	0.9997	0.7841	0.1541	0	0.0095	0.0763	0.0121
MAN	0.0018	-	0.0004	0.9909	0.3254	0	0	0	0.0008	0
HOR	0.0017	0.0011	-	0.998	0.9681	0.0018	0.0023	0	0.0174	0
CHE	-0.0018	-0.0011	-0.0013	-	1	1	0.9814	0.9893	0.9994	0.9887
CHA	-0.0394	0.0002	-0.0789	-0.0036	-	0.8519	0.8760	0.2556	0.9314	0.4809
RMB	0.0004	0.0016	0.0011	-0.0022	-0.0466	-	0.0003	0.0489	0.3379	0
MEL	0.0019	0.0019	0.0011	-0.0011	-0.524	0.0013	-	0	0.0070	0
PUY	0.0010	0.0020	0.0019	-0.0011	0.0297	0.0006	0.0020	-	0.0004	0.0002
CIS	0.0006	0.0012	0.0007	-0.0017	-0.0684	0.0002	0.0010	0.0014	-	0.0006
AYS	0.0009	0.0017	0.0015	-0.0010	0.2420	0.0012	0.0016	0.0012	0.0012	-

383 Evanno's methods showed that the likely number of K clusters was K = 2 for adaptative
384 loci (Figure S9) and K = 7 for neutral loci (Figure S10). For adaptative loci, a visual
385 inspection revealed a clear separation of individuals for REL, MAN and MEL using K=2.
386 We observed an additional cluster which appeared to correspond to individuals from CHE
387 and CHA using K = 4 (Figure 4A). For neutral loci, *Structure* showed no clear geographical
388 genetic structure (Figure 4B). Results from DAPC analyses revealed similar patterns to
389 *Structure* (Figure 5). The adaptative dataset showed that the REL, CHA and CHE were
390 separate from all other locations. The first two axis explain 44.9% of the total variation. For
391 the neutral dataset, PET, CHA and CHE overlapped greatly with the other locations and much
392 less structure overall was present, although MAN seemed to be less like the other nine
393 locations. The first two axis explain 40.5% of the total variation (Figure 5).

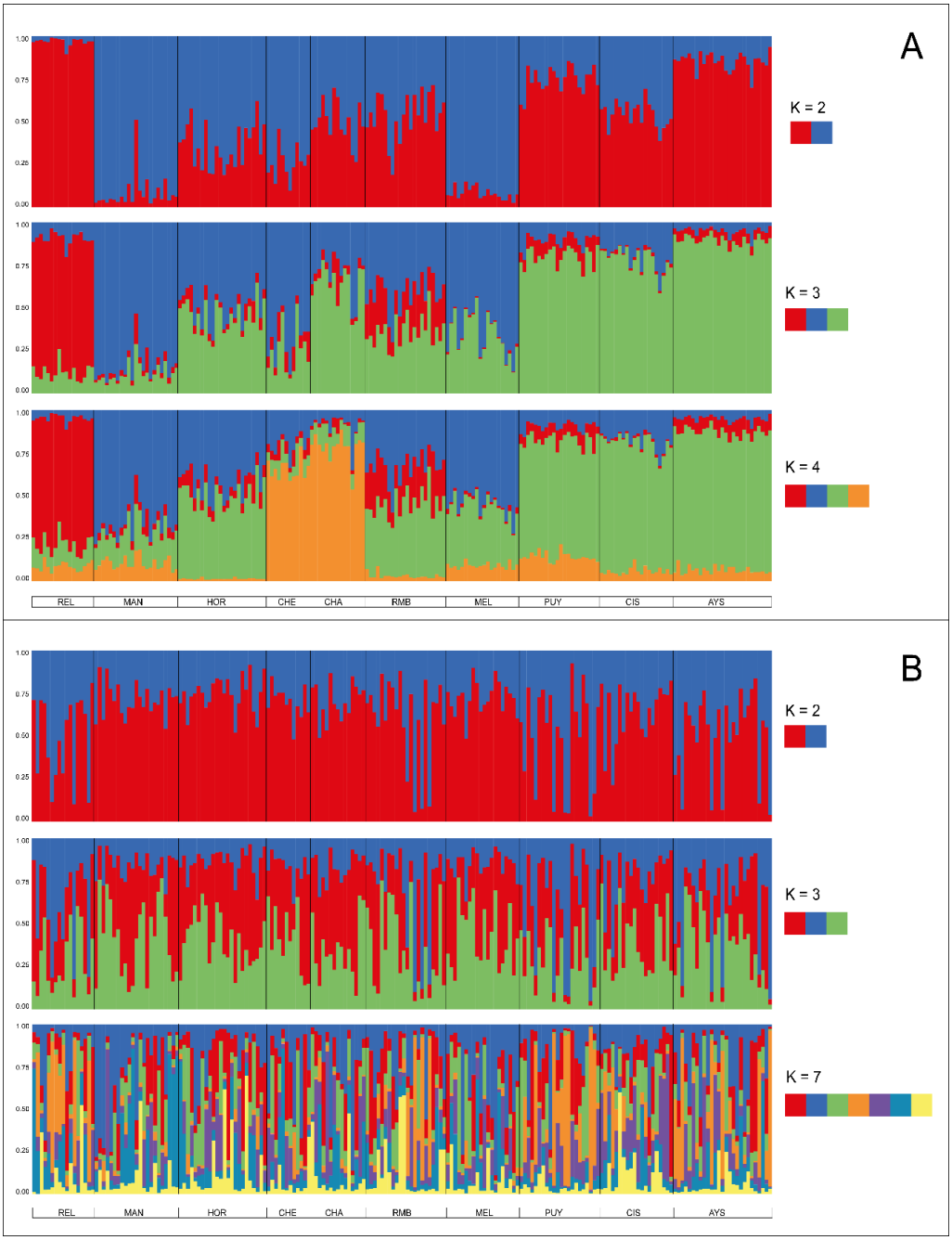
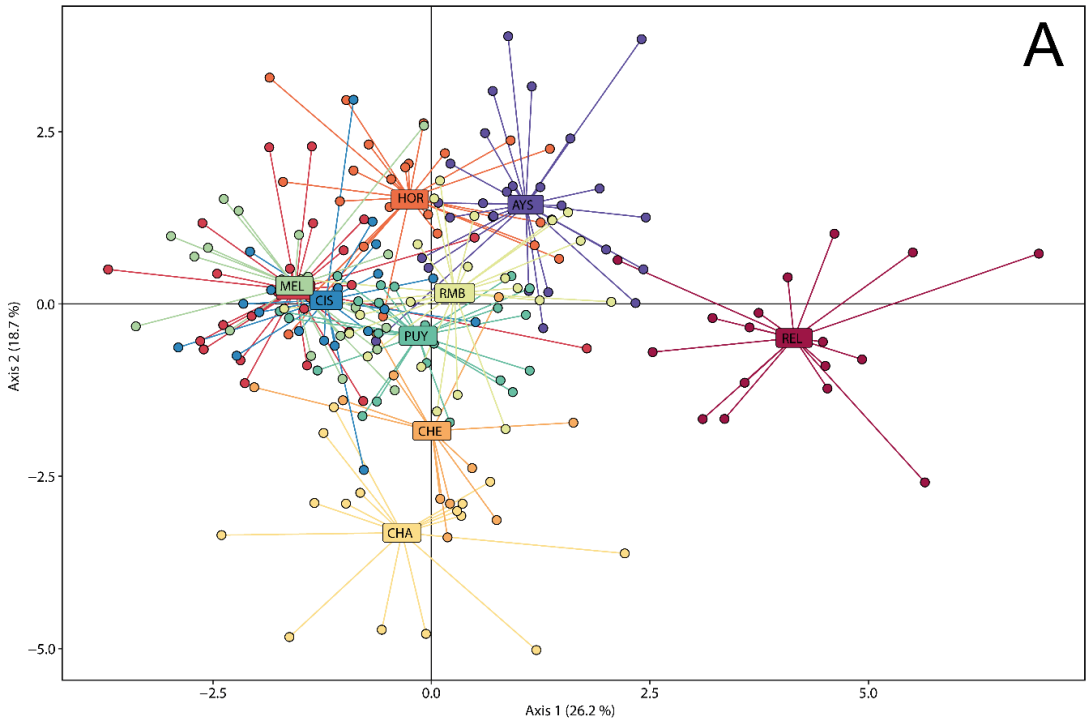
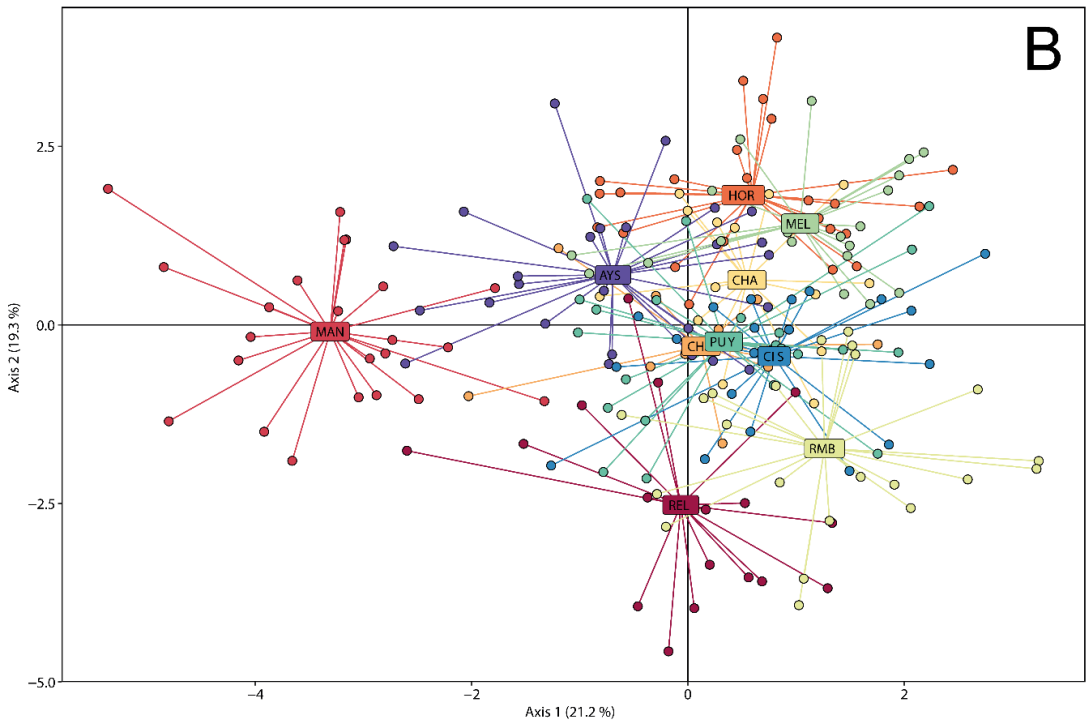


Figure 4 Results from clustering analysis of the *E. maclovinus*. Based on A) 131 putatively adaptative loci and B) 9,536 putatively neutral loci. Each vertical line represents an individual. The colors represent the proportion of inferred ancestry from

398 the K ancestral populations. Black lines separate the individuals from different locations. ΔK

399 calculated for K ranging from 1 to 10, with each K repeated tenfold.


A
HOR
AYS
MEL
CIS
RMB
PUY
REL
CHE
CHA
2.5
0.0
−2.5
−5.0
Axis 2 (18.7 %)
−2.5
0.0
2.5
5.0
Axis 1 (26.2 %)


B
HOR
MEL
AYS
CHA
MAN
CH
PUY
CIS
RMB
REL
2.5
0.0
−2.5
−5.0
Axis 2 (19.3 %)
−4
−2
0
2
Axis 1 (21.2 %)

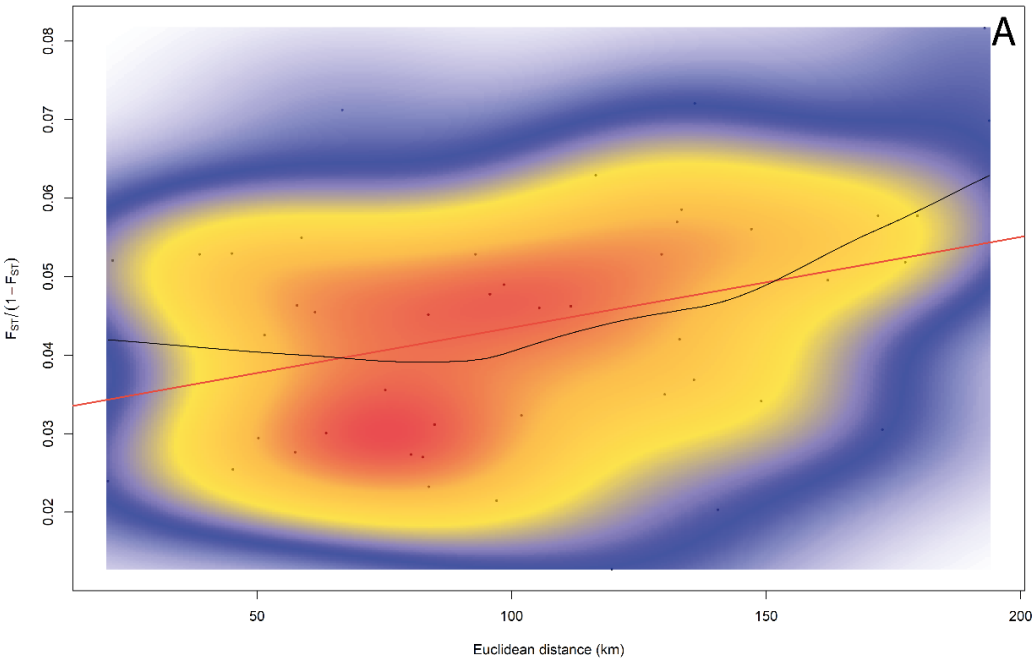
400

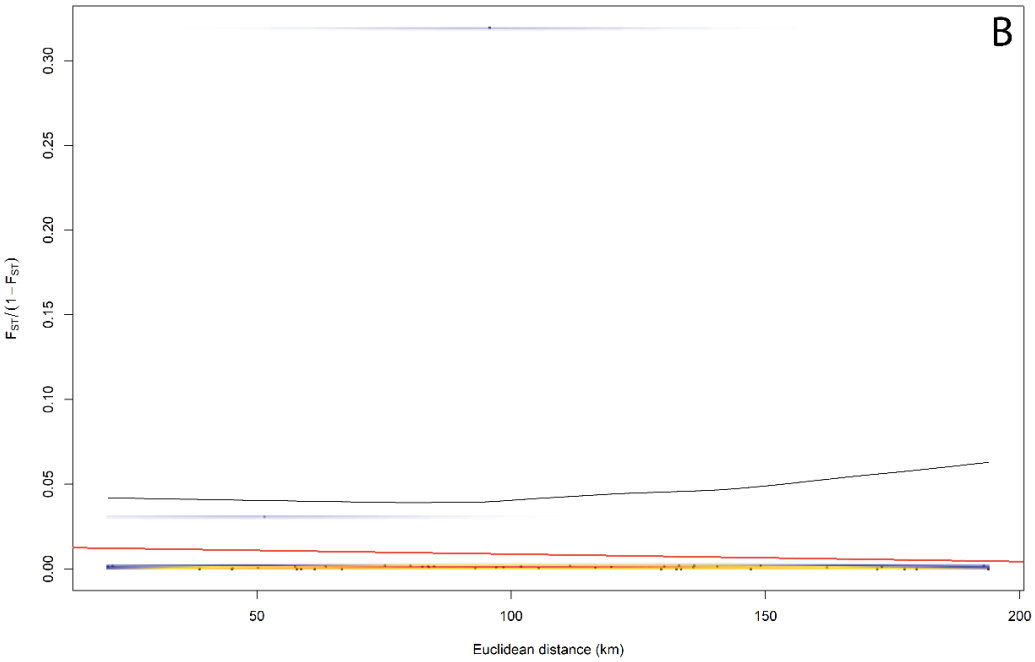
401 **Figure 5** Scatterplot of discriminant analysis of principal component (DPCA).

402 A) adaptative loci and B) neutral loci. Axes correspond to the first two discriminant functions

403 and the circles represent individuals colored-coded by sampling location.

404 The isolation by distance (IBD) analysis for adaptative loci revealed a moderate
405 significant isolation by distance pattern (Mantel test: r = 0.3458, p-value = 0.0320, Figure
406 6A), where the scatterplot of local densities of distances showed only one consistent cloud
407 of points, indicating a continuous cline of genetic differentiation. For neutral loci, we found
408 no detectable relationship between geographic distance and genetic distance (Mantel test: r
409 = -0.0435, p-value = 0.5760, Figure 6B).


A
0.08
0.07
0.06
0.05
0.04
0.03
0.02
$F_{ST}/(1-F_{ST})$
50
100
150
200
Euclidean distance (km)


B
0.30
0.25
0.20
0.15
0.10
0.05
0.00
$F_{ST}/(1-F_{ST})$
50
100
150
200
Euclidean distance (km)

410

411 **Figure 6** Isolation-by-distance IBD among locations. A) 131 adaptative loci and B)
412 9,536 neutral loci. Local density of points plotted using two-dimensional kernel density
413 estimation. The black line is the trend estimated by Loess regression red line indicates the
414 linear regression. Color gradient represents density of observations (low = blue, red = high).

3.4 Additive polygenic scores (APS)

The correlations between additive polygenic scores (APS) and environmental variables were all significant ($p < 0.001$). The R^2 values ranged from 0.29 for maximum temperature to 0.26 for both maximum salinity and minimum oxygen concentration. For all environmental variables, APS decreased with increasing temperature, salinity, and oxygen levels, respectively (Figure 7). The correlation between APS and environmental variables was best represented by linear models, except for maximum temperature, which fitted better with a quadratic model (Figure 7). The significance and R^2 values of the correlations calculated for mean and minimum temperature, salinity, and oxygen concentration yielded similar results, albeit with slight differences among subsets (Figure S11-S13).

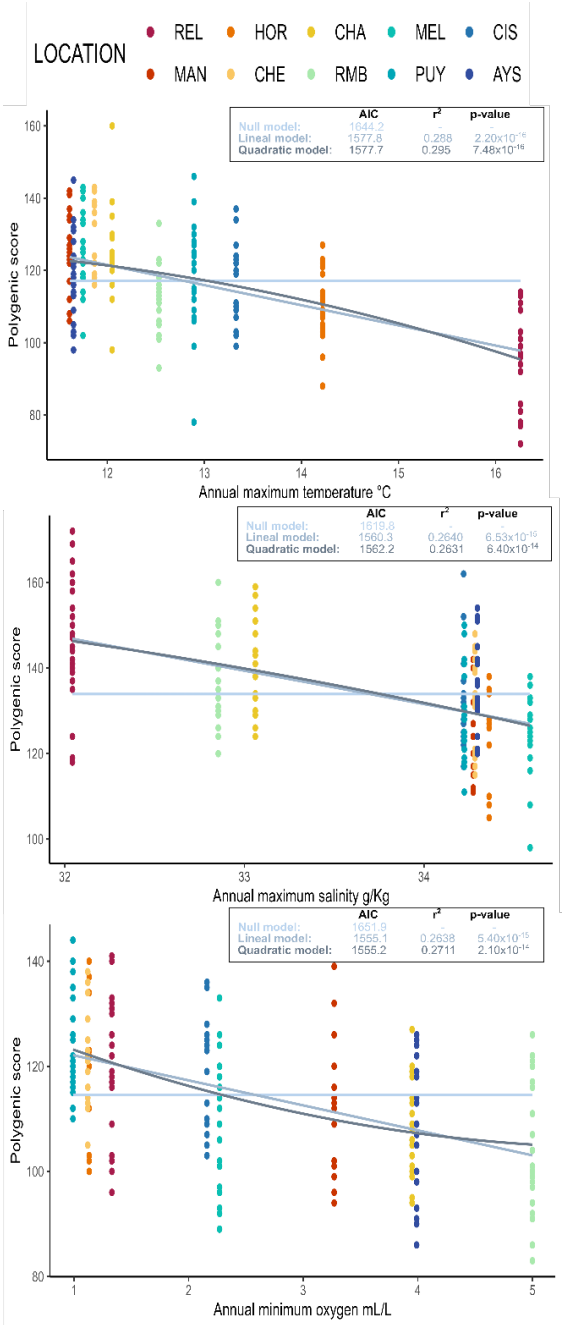
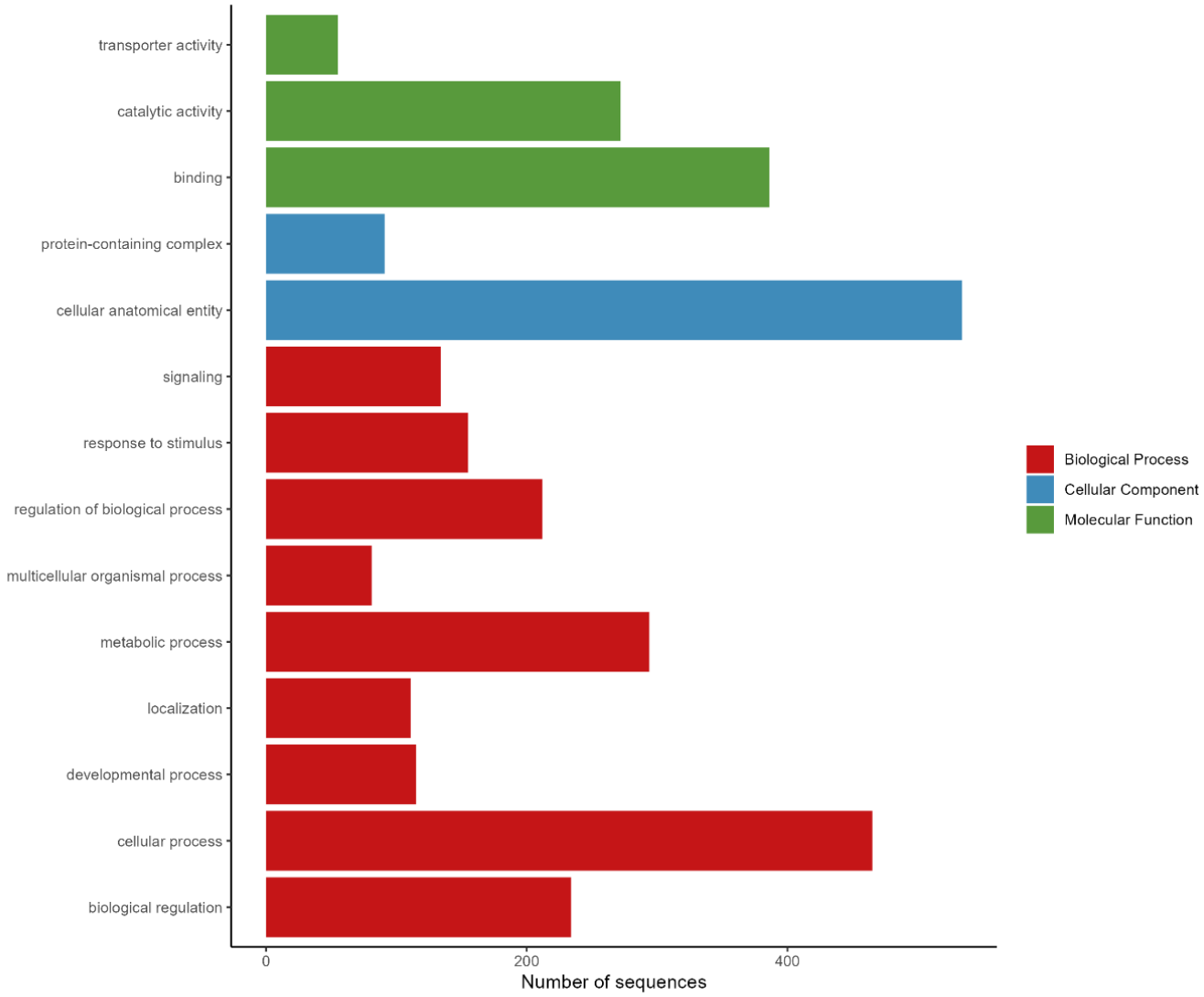


Figure 7 Correlations between additive polygenic scores (APS) based on A) Annual maximum temperature B) Annual maximum salinity and C) Annual minimum oxygen and 131 putative adaptative loci. Correlation coefficient (R^2) and p-values and AIC are presented for each variable.

434 **3.5 Gene ontology of candidate genes**

435 The BLASTx analysis of the contigs containing the 131 adaptative loci shared by PGD
436 and GEA against bony fish genomes resulted in significant hits (E-value $\leq 1x10^{-5}$) for 790
437 fish species. The BLASTx similarity results showed that 51 of the 131 contigs containing
438 adaptative loci corresponded to known proteins in the non-redundant (NR) database (E-value
439 $1e^{-5}$), of which 36 contigs were functionally annotated. The functional categorization of the
440 annotated sequences involved biological process, cellular components, and molecular
441 function (Figure 8).

442



443 **Figure 8** Putative functional categorization and distribution according to Blast2GO.

444 A second BLASTx analysis with all 2,425 putative loci detected 512 contigs that were
445 functionally annotated. Of these 512 sequences, three were already known to potentially play
446 a role in the local adaptation (Table S1). The sequences of the *SNP 6291_214* and *44791_149*
447 and belong to the solute carrier (SLC) family. SLC genes encode transmembrane transporters
448 for inorganic ions, amino acids, neurotransmitters, sugars, purines and fatty acids, and other
449 solute substrates (Dorwart *et al.* 2008). The SNP 42_59 is located in the vtgC (13489) gene,
450 which is a member of the vitellogenins (Vtg) family. In egg-laying vertebrates such as fish,
451 estrogens activate the hepatic synthesis of vitellogenin (Vtg) (Sumpter & Jobling 1995; Brian
452 *et al.* 2008).

453 **4 Discussion**

454 This study provides comprehensive evidence for genomic signatures of local adaptation,
455 of *Eleginops maclovinus* to heterogeneous environmental conditions in Northern Patagonia.
456 We conducted a seascape genomics study using 11,961 RAD-sequencing markers and
457 detected 2,164 putative adaptative loci through population genetic differentiation (PGD) and
458 genotype-environment association (GEA) approaches. Using 131 loci shared by PGD and
459 GEA, we observed that the putative adaptive loci collectively exhibit a highly significant
460 association with various environmental variables, indicating polygenic selection.
461 Additionally, we found contrasting patterns of genetic variation in neutral and adaptative loci
462 that reinforce the possible local adaptation among high levels of gene flow. Subsequently,
463 we detected one gene related to thermal adaptation and two genes that play a role in the
464 osmoregulation. In the following, we describe how our study contributes to understanding
465 the role of environmental heterogeneity as a driver of spatial patterns of putative adaptive
466 genetic variation that could influence the local adaptation in *E. maclovinus*. Furthermore, we
467 discuss methodological issues about software for detecting putative adaptative loci. Finally,
468 we propose groups based on adaptative divergence for creating management units.

469 **4.1 Evidence for spatially divergent selection**

470 Evidence suggests thermal adaptation *E. maclovinus*, which juveniles exhibit
471 significant eurythermal capacity, tolerating temperatures between 4 and 20°C (Vanella *et al.*
472 2012; Oyarzún *et al.* 2018, 2021). Short-term exposure to 4°C may reduce fitness (Vanella
473 *et al.* 2012), while long-term exposure to 15-20°C elevates metabolism with tissue-specific

474 modulation, without adverse physiological effects. Conversely, temperatures exceeding 25°C
475 detrimentally impact physiology, growth, food intake, and energy substrates, mainly
476 triglycerides (Oyarzún *et al.* 2021). These studies focus on juveniles; thus, temperature
477 effects on early-stage adaptation remain uncertain for this species. Considering *E. maclovinus*
478 spawns in spring (Brickle *et al.* 2005), higher temperatures could expedite maturation (e.g.
479 Scott & Pankhurst 1992; Shimizu 2003; Hokanson 2011). Temperature affects reproductive
480 processes' thermal inhibition (Gillet *et al.* 1996; Tveiten *et al.* 2001; Okuzawa *et al.* 2003;
481 Soria *et al.* 2008; Pankhurst & King 2010), including egg mortality (Van Der Kraak &
482 Pankhurst 1997; Gagliano *et al.* 2007; Janhunen *et al.* 2010), embryonic development rate
483 (Pauly & Pullin 1988; Rombough 1997; Smith *et al.* 2015), and larval fish metabolism,
484 growth, and development (Houde 1989; Blaxter 1992; Benoît *et al.* 2000). Future research
485 should examine temperature-induced shifts in selective mortality in early stages of *E.*
486 *maclovinus*. Additionally, temperature may influence sexual inversion in protandrous
487 hermaphrodites, as proposed by Baroiller et al. (1999). For instance, elevated temperatures
488 increased gonadal aromatase levels, promoting female proportion in *Lates calcarifer,* another
489 protandrous hermaphrodite (Athauda *et al.* 2012). This latter, although not identified in *E.*
490 *maclovinus*, can be taken as a research question for future studies. The detection of
491 potentially adaptive loci associated with temperature supports molecular adaptation via gene-
492 related heterogeneity and gene duplication events, likely preserved in response to thermal
493 fluctuations. Unlike most Antarctic notothenioids, *E. maclovinus* blood shows high
494 hemoglobin (Hb) multiplicity, with one cathodal (Hb C) and two anodal components (Hb 1,
495 Hb 2) (Coppola *et al.* 2010). This Hb diversity may facilitate oxygen transport adaptation to

496 environmental changes and metabolic demands (Weber 1990; di Prisco & Tamburrini 1992;
497 Feuerlein & Weber 1994; Weber *et al.* 2000; Fago *et al.* 2001). The maintenance of multiple
498 Hbs in response to temperature differences in temperate waters, significantly larger than in
499 Antarctica, is likely (Coppola *et al.* 2010; di Prisco *et al.* 2012). Moreover, the absence of
500 antifreeze-glycoprotein (AFGP) coding sequence in *E. maclovinus* indicates no adaptation to
501 near-freezing temperatures (Cheng *et al.* 2003).

502 Evidence suggests salinity acts as a selective agent driving genetic differentiation in *E.*
503 *maclovinus* populations. Canales-Aguirre et al. (2022) identified associations between
504 adaptive loci and environmental variables (salinity, oxygen, primary production, current
505 velocity) in *E. maclovinus*. Juveniles are considered euryhalines, adapting to salinities
506 between 5 and 45 PSU (Vargas-Chacoff *et al.* 2014, 2015a). An inverse relationship between
507 the trypsin chymotrypsin−1 ratio (T/C ratio) and salinity was observed (Vargas-Chacoff *et*
508 *al.* 2015b). The T/C ratio, linked to growth and nutritional status (Rungruangsak-Torrissen
509 *et al.* 2006; Rungruangsak-Torrissen 2007), suggests better food utilization in low salinity,
510 enhancing physiological energy. It is possible that food utilization and efficiency is better in
511 *E. maclovinus* acclimated to low salinity, leading not to better growth, but to increased energy
512 available for physiological processes such. Osmoregulation in low salinity consumes energy,
513 affecting growth (Vargas-Chacoff *et al.* 2015b). Euryhaline fish adapt to varying osmotic
514 challenges (Laverty & Skadhauge 2012). Climate change, habitat degradation, and
515 anthropogenic activities increase salinity stress (Kültz 2015). Climatic models for Northern
516 Patagonia predict reduced precipitation and increased temperature, affecting hydrological
517 regimes and salinity (Aguayo *et al.* 2019). The salinity variability in microenvironments can

518 have multiple impacts on reproduction, including DNA damage from salt stress or oxidative
519 stress (Dowling & Simmons 2009), sperm motility (Beirão *et al.* 2018), incomplete egg
520 binding (Herberg *et al.* 2018), disrupted egg activation (Ginsburg 1963) and egg penetration
521 issues (Yanagimachi *et al.* 2017), and cause problems during DNA recombination (O, Wai-
522 sum *et al.* 2006).

523 Our results indicate oxygen concentration exerts selective pressure on *E. maclovinus.*
524 Environmental oxygen variability has been crucial in fish evolution, influencing anatomical,
525 behavioral, and physiological adaptations for oxygen acquisition and delivery (Nikinmaa &
526 Rees 2005). Fish have evolved to balance oxygen delivery while avoiding toxic reactive
527 oxygen species (Taylor & McElwain 2010; Dong *et al.* 2011). They exhibit diverse
528 hemoglobin structures and functions for oxygen transport (Powers 1974; Jensen & Weber
529 1985; Weber 1990; Jensen *et al.* 1998). Hemoglobin diversity in fish, including E.
530 maclovinus, likely results from selection pressures like low oxygen levels(Nikinmaa & Rees
531 2005). Structural and functional analyses of *E. maclovinus* hemoglobin (Hb1Em) showed
532 higher O_2 affinity compared to *T. bernacchii,* indicating differences in temperature sensitivity
533 and O_2 release between sub-Antarctic and Antarctic notothenioids (Coppola *et al.* 2012).
534 Silva & Vargas (2014) identified hypoxic zones in Northern Patagonia's fjords (1.1–2.0 mL
535 L-1); we emphasize the importance of studying the ability of to adapt to hypoxic conditions
536 in *E. maclovinus* until now unclear.

537 In this study, we identified putative adaptive loci associated with the concentration of
538 nitrate, phosphate, and silicate. Although there is no direct relationship between nutrient

concentration and adaptive capacity in *E. maclovinus,* the nutrients could be a proxy for primary productivity. The productivity cycle of Patagonian channels and fjords has been divided into two seasons: a productive season characterized by a marked spring diatom bloom followed by pulsed productivity events dominated by a sequence of different phytoplankton species, and then a non-productive season that predominantly features small phytoplankton cells (Iriarte *et al.* 2007; Czypionka *et al.* 2011; Montero *et al.* 2011; Paredes & Montecino 2011). During the productive season and in the absence of persistent hydrodynamic phenomena that force nutrient replenishment in the euphotic zone, the supply of nitrate and phosphate to the well-lit surface layer would be the chief limiting factor for phytoplankton growth (Pizarro *et al.* 2000; Iriarte *et al.* 2007). The increased freshwater discharge into the Puyuhuapi channel during non-productive season coincides with increases in the concentrations of silicic acid and dissolved organic matter concentrations, as well as an increase in underwater light attenuation (Montero *et al.* 2017). A GEA analysis of Canales-Aguirre (2022) provided evidence for local adaptation associated to primary production on *E. maclovinus* likely connected to food availability (Gove *et al.* 2016; Fox *et al.* 2018). Similarly, multiple studies detected putative adaptive loci associated with chlorophyll (indicator of primary productivity), for example Boulanger *et al*. (2022) with *Mullus surmuletus,* Cayuela *et al*. (2020) with *Mallotus villosus,* Maselko et al. (2020) with *Sebastes alutus* and Diopere *et al.* (2017) with *Solea solea*.

4.2 **Additive Polygenic Scores (APS)**

For the Additive Polygenic Scores (APS) (Gagnaire & Gaggiotti 2016; Babin *et al.* 2017), we observed a strong correlation between polygenic scores and maximum temperature

561 and salinity, and minimum oxygen. This suggests that *E. maclovinus* populations might be
562 locally adapted to these environmental variables through a polygenic architecture. As we
563 highlighted in the previous section (See 4.1.), we detailed the impact of these environmental
564 variables on fish survival and development.

565 We found negative correlations between individual APS and all environmental
566 variables, which could indicate directional selection, where different alleles are maintained
567 in distinct environments with a relatively constant selection force along the environmental
568 gradient. Specifically, the relationship between APS and maximum temperature was best
569 represented by a quadratic model, suggesting stabilizing selection at intermediate
570 temperatures. However, caution is advised with this conclusion as the difference in AIC
571 between the linear and quadratic models was minimal (AIC_{linear} = 1577.8, $AIC_{quadratic}$ =
572 1577.7).

573 The impact of temperature on additive genetic variation agreed with observations in
574 the adaptive population structure membership plots, where the REL population in the
575 northernmost latitudinal range with warmer temperatures separates from the other nine
576 populations. For salinity and oxygen concentration, the linear model better explained the
577 relationship with APS, indicating relatively constant selection along the salinity and oxygen
578 gradients. These results are consistent with the observed adaptive population structure, where
579 the CHE and CHA populations differentiate from the rest, with the CHE population exposed
580 to higher oceanic salinities. Lastly, the minimum oxygen concentration is critical for fish
581 reproduction and development, as hypoxia can delay embryonic development and hatching

582 (Richards 2009; Wu 2009). However, it remains unknown how hypoxia affects the genetic
583 expression and reproductive development of *E. maclovinus*.

584 While the APS approach has potential for evaluating the cumulative effect of
585 potentially adaptive loci in complex environments, it has limitations. Without a reference
586 genome, we cannot rule out the possibility that some potentially adaptive loci are linked. An
587 annotated *E. maclovinus* genome would allow us to identify matches between adaptive loci
588 and selected genomic regions, providing insights into specific genes involved in local
589 adaptation (Manel *et al.* 2016). Additionally, comparing genotypic variation in potentially
590 adaptive loci with individual phenotypic data is necessary to establish genotype, phenotype,
591 and fitness associations (Barrett & Hoekstra 2011).

592 Our results suggest that maximum temperature, maximum salinity, and minimum
593 oxygen concentration may drive spatially variable selection in *E. maclovinus* populations.
594 Temperature, salinity, and oxygen concentration impact the environmental gradient
595 experienced by eggs and larvae (Holliday 1969; Blaxter 1992; Brickle *et al.* 2005; Wu 2009;
596 Lehtonen & Kvarnemo 2015), potentially influencing local adaptation. Future research
597 should aim to detect molecular signatures of selection within a multilocus quantitative
598 genetics framework to delve deeper into the genetic architecture of polygenic adaptive traits.

599 **4.3 Finding functional genomics of candidate genes for local adaptation**

600 Using BLASTx to identify genes linked to adaptation through a gene ontology
601 approach (Haasl & Payseur 2016; Manel *et al.* 2016), we found a total of 2,425 potentially

602 adaptative loci with 131 loci shared by PGD and GEA, only 512 and 36 loci were successfully
603 annotated, respectively. In the 131 adaptative loci, only 39.69% of loci matched known
604 sequences in the public database, highlighting the limitation of RAD sequencing in
605 identifying candidate genes without a reference genome (Davey *et al.* 2013; Andrews *et al.*
606 2016; Catchen *et al.* 2017; McKinney *et al.* 2017a). Both adaptive datasets revealed loci
607 linked to life-history traits, including thermal adaptation and osmoregulation.

608 Focusing on life history traits, we identified five key genes: *opa1, Stx16, FMVIA, foxj1b,*
609 and *Lamc3*. The gene *opa1* regulates mitochondrial fusion and fission, preventing Cyc-c
610 leakage and inducing apoptosis, which is linked to kidney injury (Song *et al.* 2017; Yingjie
611 *et al.* 2019; Herkenne *et al.* 2020; Wang *et al.* 2020; Cui *et al.* 2021; Li *et al.* 2022). The
612 *Stx16* is involved in the development of inducible defense structures in *Daphnia pulex* under
613 predation stress (Spanier *et al.* 2010). High *FMVIA* expression in the retina of striped bass
614 suggests a role in retinal motility (Breckler *et al.* 2000). The *foxj1b* gene is essential for
615 normal laterality in zebrafish embryos, affecting heart and organ positioning (Tian *et al.*
616 2009). Finally, *Lamc3* plays a role in the development of the parachordal chain in zebrafish
617 (Eve & Smith 2017).

618 Expanding our search to adaptive loci, we identified three key genes: *vtg3* (linked to
619 temperature adaptation) and *slc39a6, slc8a2a* (associated with salinity adaptation). *Vtgs* are
620 specialized large lipid transfer proteins produced by the liver and transported to the ovary
621 (Babin *et al.* 2007). The *Vtg* family includes type-I (*Vtg 1, 4, 5, 6,* and *7*), type-II (*vtg2* and
622 *vtg8*), and type-III (*vtg3*) (Yilmaz *et al.* 2018). Despite being a minor and divergent form,
623 *vtg3* significantly contributes to zygote and early embryo development (Yilmaz *et al.* 2019).

624 Increased temperature elevates estrogen-induced *vtg* expression in juvenile *Salmo trutta*
625 (Körner *et al.* 2008), suggesting a similar role for *vtg3* in *E. maclovinus* related to thermal
626 adaptation. The gene *slc39a6 (LIV-1)* maintains zinc homeostasis, essential for cellular
627 growth and differentiation (Taylor & Nicholson 2003). Low salinity increases *slc39a6*
628 expression in tiger puffer (*Takifugu rubripes*) (Jiang *et al.* 2020), and intracellular zinc
629 homeostasis controls T-cell survival in zebrafish by inhibiting apoptosis (Zhao *et al.* 2019).
630 The *SLC8* gene family (*NCX*) maintains cellular Ca2+ homeostasis (Khananshvili 2013).
631 *Slc8a2a*, an isoform identified in zebrafish, is key for Ca2+ excretion by seawater fish
632 kidneys, with upregulated transcription during seawater acclimation (Liao *et al.* 2007; Islam
633 *et al.* 2011). These genes indicate positive selection associated with salinity in *E. maclovinus*
634 in Northern Patagonia.

635 We did not identify genes whose functions are supposed to be affected by the
636 concentration of oxygen or nutrients (nitrate, phosphate and silicate). However, many outlier
637 sequences did not have BLASTx results and may represent important proteins that have not
638 been adequately annotated in fish to date.

639 **4.4 Comparing approaches for detecting genomic signal of selection**

640 The outcomes from the ten programs varied widely in terms of the number and identity
641 of adaptative loci detected in this study. Such variations among methods were expected since
642 their algorithms and prior assumptions considerably differ. Combining population genetic
643 differentiation (PGD) and genotype-environment association (GEA) approaches to detect
644 adaptative loci of local adaptation not only reduces false-positive discoveries, but also

645 maximizes our chances of detecting potential signals of selection (François *et al.* 2016).
646 Overall, we found that GEA approach identified more adaptative loci (2,164 loci) than PGD
647 approach (392 loci). These outcomes are in agreement with a simulation study demonstrating
648 that the GEA approach has more power to detect loci under divergent selection than PGD
649 approach (de Villemereuil *et al.* 2014), which is not surprising given that GEA uses multiple
650 environmental variables (six variables in our case) to depict signals of selection. This trend
651 has been reported in multiple studies focused on detecting signs of local adaptation in marine
652 populations (Benestan *et al.* 2016; Flanagan *et al.* 2016; Dalongeville *et al.* 2018; Bekkevold
653 *et al.* 2020; Cayuela *et al.* 2020; Segovia *et al.* 2020).

654 Inside of PGD approach, *fsthet* detected the largest number on putative adaptative loci
655 (n= 313 loci). This software generates smoothed quantiles for the F_{ST}–heterozygosity
656 relationship from the empirical distribution (Flanagan & Jones 2017). Using an empirical
657 distribution itself has the advantage of not assuming a particular distribution or model of
658 evolution. For example, *FDIST2* and *LOSITAN* used an idealized island model, which would
659 not be appropriate considering the geography of Northern Patagonia. Besides, this feature is
660 appealing because the F_{ST}–heterozygosity distribution changes based on many potentially
661 unknowable features of population structure (Flanagan & Jones 2017). Though, *fsthet*
662 decreased the average proportion of false positives compared with *LOSITAN*, we did not find
663 any studies that compared the effectiveness of *fsthet* with respect to other software that
664 considers the hierarchical population structure, such as *Arlequin*. The *fsthet* program is not
665 recommended when migration rates are low because the neutral loci saturate the full
666 distribution of potential F_{ST}–heterozygosity values (Flanagan & Jones 2017). In our case, the

667 relative migration rates showed high gene flow between locations of *E. maclovinus* (Canales-
668 Aguirre *et al.* 2022). The *Arlequin* identified 270 loci (200 shared with *fsthet*). However,
669 results of a simulation study showing that *Arlequin* consistently found more outliers and had
670 the highest type I and type II errors in their simulation scenarios in compared with other
671 methods such as *BayeScan*. Simulated data suggested that *Arlequin* does not perform well in
672 situations where adaptive variation contrasts with patterns of neutral variation, likely due to
673 the effect of hierarchical grouping based on neutral markers (Narum & Hess 2011). The
674 *PCAdapt* was the more conservative (n= 15 loci), whereas *BayeScan* and *OutFLANK* did not
675 detect any outliers. Population structure and the presence of admixture individuals affect the
676 power of identifying adaptive loci with *Bayescan* more than *PCAdapt* (Luu *et al.* 2017). This
677 latter, agreed with our data where we found admixture individuals in the whole SNPs
678 database. The presence of mixed individuals is supported by the high relative migration rates
679 estimated by Canales-Aguirre *et al.* (2022). Additionally, *BayeScan* could decrease power
680 with few populations (n <30 Aguirre-Liguori *et al.* 2020) and weak selection, when
681 populations deviate from the island model of migration and/or in cases of smaller sample size
682 (Foll & Gaggiotti 2008; Narum & Hess 2011).

683 Inside the GEA approach, *LFMM* detected the largest number of potentially adaptive
684 loci (n= 1,785 loci). Considering that *LFMM* identified 14.92% of the loci as adaptive, it is
685 likely that we will find false positives among them. The main reason is that geographic and
686 demographic processes can lead to patterns that mimic those observed due to selection. In
687 fact, de Villemereuil *et al*. (2014) found high rates of false discovery in some scenarios with
688 complex, hierarchical structures and polygenic selection; although showed that *LFMM*

689 provides the best compromise between detection power and error rates in situations with
690 complex hierarchical neutral genetic structure and polygenic selection. However, Frichot et
691 al. (2013) found that *LFMM* has low rates of false positives and negatives and that it performs
692 slightly better than *Bayenv* in detecting weak selection. Finally, Lotterhos & Whitlock (2015)
693 showed that *LFMM* is quite robust to various sampling designs and underlying demographic
694 models. The *MSOD* detected considerably fewer potentially adaptive loci (n=377 loci).
695 However, the main advantage of this program is that using Moran spectral randomization
696 (MSR) for significance testing, avoids the inflation of type I errors due to spatial
697 autocorrelation, whether resulting from gene flow or the spatial structure of the
698 environmental factors that induce selection (Wagner & Dray 2015; Wagner *et al.* 2017).
699 Moreover, spatial outlier detection worked best for scenarios of IBD, even in the presence of
700 large-scale spatial genetic structure (Wagner *et al.* 2017) as in this study. *RDA* detected the
701 fewest number of putative adaptive loci (n=83 loci) compared with *LFMM* and *MOSD*. The
702 main difference is the multivariate approach of *RDA*, while *LFMM* and *MSOD* are univariate,
703 meaning that they test one genetic marker at a time, and may also test only one environmental
704 predictor at a time (Rellstab *et al.* 2015); *RDA* identifying covarying loci associated with the
705 multivariate environmental variables (Legendre & Legendre 2012). The decrease in the
706 number of loci detected by *RDA* is because it restricts the number of environmental variables
707 tested based on the variance inflation factor. In the case of univariate programs, we tested six
708 environmental variables with four statistics for each but restricted to one statistic per
709 environmental variable for *RDA*, limiting the number of possible correlations. However,
710 simulated data showed that *RDA* has higher true-positive and lower false-positive rates

711 compared to *PCAdapt* and *LFMM* (Forester *et al.* 2018; Capblancq *et al.* 2018). Furthermore,
712 *RDA* has one important limitation: it models linear relationships among response and
713 predictors, meaning that nonlinear relationships will not be detected (Capblancq & Forester
714 2021). Finally, we did not detect any putative adaptive loci using the *BayeScEnv* and
715 *Samβada* programs. Considering that *BayeScEnv* produces fewer candidate loci than
716 *BayeScan* (de Villemereuil & Gaggiotti 2015), it is to be expected that if *BayeScan* did not
717 detect any loci *BayeScEnv* either. To date, we have not identified any studies that compare
718 the statistical power or control of FDR of *BayeScEnv* with respect to other programs.
719 Therefore, we cannot conclude whether *BayeScEnv* is more or less sensitive to certain
720 characteristics such as hierarchical population structure, isolation by distance, or strength of
721 selection. Due to the recentness of the *Samβada*, we did not identify comparative studies that
722 allow us to evaluate the advantages and disadvantages of this with respect to other GEA
723 approaches.

724 Continued testing and comparing PGD and GEA approaches is needed in simulation
725 frameworks that include more population structure, multiple selection surfaces and genetic
726 architectures that are more complex than the multilocus selection response modelled here.
727 Overall, we highlight the importance of combining PGD and GEA approaches that consider
728 multiple scenarios of adaptive variation, to diminish type I and II errors and avoid conclude
729 erroneously.

730 **4.5 Implications for management and conservation**

731 The 11,961 filtered loci genotyped for 202 individuals (the largest population genomic
732 data set reported so far for *Eleginops maclovinus*) offers high resolution for creating
733 management policies and decisions, with respect to patterns of adaptive genetic variation.
734 Although neutral loci did not reveal a clear genetic structure among locations, analysis of
735 131 shared adaptive loci indicated genetic differentiation separating the populations into four
736 groups: REL, CHE, CHA, and a fourth group comprising the remaining seven sampling
737 locations. Note that all neutral pairwise F_{ST} values were close to zero, similar to showed by
738 Canales-Aguirre *et al*. (2022) and also from fish and other marine species (Nayfa & Zenger
739 2016; Asaduzzaman *et al.* 2019b; a; O'Leary *et al.* 2021). The interpretation of extremely
740 small F_{ST} is not straightforward (Waples & Gaggiotti 2006; Conover *et al.* 2006; Waples *et*
741 *al.* 2008), meaning it is unclear what the implications of the patterns of weak neutral
742 differentiation may be for demographic connectivity of subpopulations. Nevertheless, the
743 signals of putatively adaptive genetic differentiation between the ten locations were
744 significant. Our study demonstrates how the environment shapes the distribution of adaptive
745 genetic variation across space for *E. maclovinus*; showing that REL, CHE and CHA retain
746 adaptive characteristics different from the rest. This can have important implications for
747 prioritizing sites for protection, where the level of adaptive genetic variation could be an
748 indicator of the evolutionary resilience of populations (Bonin *et al.* 2007; Sgrò *et al.* 2011).
749 Moreover, understanding the spatial distribution of putatively adaptive genetic variation can
750 inform the selection of specific sites for protection within marine reserves to maintain the
751 adaptive potential and evolutionary resilience of wild populations faced with environmental

752 change (von der Heyden 2017). The adaptive divergence reported here appears to be
753 underpinned by a variation in seawater temperature and oxygen concentrations in the
754 Northern Patagonia (Iriarte *et al.* 2014; Torres *et al.* 2014; Pérez-Santos *et al.* 2014; Silva &
755 Vargas 2014) and should affect traits relevant for the survival, growth and reproduction of *E.*
756 *maclovinus*. The complex patterns of population structure and putatively adaptive diversity
757 among *E. maclovinus* locations that we showed here are not recognized in current *E.*
758 *maclovinus* management units. To date, there is no management plan for *E. maclovinus* in
759 Chile, only regulations for the type of fishing gear used. Future research could use neutral
760 and adaptative datasets to determine management units using adaptative (e.g., population
761 adaptative index, adaptative score) and neutral (e.g., expected heterozygosity, local
762 differentiation) metrics as suggested by Xuereb et al. (2021). In the Chilean Northern
763 Patagonia, temperature and heat content are predicted to increase and freshwater river inputs
764 are predicted to decrease due to climate change (Aguayo *et al.* 2019). As such, the spatial
765 patterns of genetic variability observed in this study can be information conservation
766 planning decisions by identifying for protection populations containing higher levels of
767 segregating polymorphisms associated with environmental conditions (e.g., temperature and
768 salinity) that are prone to change in the future.

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