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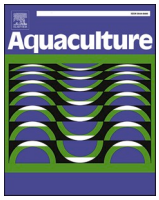

First evidence of fine-scale adaptive genetic structure in farmed populations of *Mytilus* mussels

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ABSTRACT

In Chile, the world's second-largest mussel producer, farming of the edible *Mytilus* mussels relies on the collection of wild larvae and its subsequent transfer to high-density grow-out areas, primarily in northern Patagonia. Previous studies have described the species as a highly diverse panmictic unit with low spatial genetic differentiation. The genetic diversity of farmed populations and the influence of environmental heterogeneity, however, remain unexplored. This study examines the genetic structure of cultivated and wild populations in Chaparano and Bahía Ilque, two nearby locations (<50 km apart) in northern Patagonia, a region characterized by strong vertical and horizontal environmental gradients. We obtained 97,722 SNPs using Genotyping-by-Sequencing (GBS) from 91 individuals. Our results confirm low spatial genetic structure in neutral loci, suggesting high connectivity among wild populations. However, we identified 18 putatively adaptive SNPs, indicating subtle local adaptation. Notably, the farmed population in Chaparano exhibited high genetic differentiation from the adjacent wild population, likely linked to differences in maximum temperature and salinity range. These findings suggest that aquaculture conditions can impose selective pressures, even when farmed populations experience similar oceanographic conditions to wild populations. This study provides the first evidence of fine-scale adaptive differentiation in *Mytilus* mussels, highlighting the role of environmental variability in shaping genetic structure. Understanding these dynamics is crucial for the sustainability of mussel aquaculture as a social-ecological system, ensuring the conservation of genetic diversity and long-term resilience.

1. Introduction

The cultivation of the Chilean edible mussel *Mytilus chilensis* Hupé, 1854 has experienced significant growth, reaching annual landings of around 400,000 tons (SERNAPESCA, 2000–2024), establishing Chile as one of the world's leading producers and exporters of mussels (FAO, 2024). Additionally, the sector forms a sensitive social-ecological system in Chile; production is concentrated in the northern Patagonia region and maintains approximately 17,000 direct and indirect jobs at the national level (Dresdner et al., 2017). Farming relies entirely on the capture of planktonic wild spat (juveniles) spawned from wild populations, which are collected in suspended collection systems (Contreras and Godoy, 2021; Molinet et al., 2021). Spat collection takes place in a limited number of Management and Explotation Areas for Benthic Resources (MEABRs) and Aquaculture concessions located in low-salinity zones, such as the Reloncaví Fjord and the eastern coast of the Chiloé Inland Sea (Fig. 1). Once spat reach a size of 1.5 to 3 cm—approximately two months after settlement on collectors—they are transferred to on-growing centers located in different areas, primarily near Chiloé Island and the western coast of the Reloncaví Sound. Juveniles attached to collectors are placed in cotton mesh sleeves to facilitate reattachment to a rope substrate which is then deployed on ropes on longlines (Contreras and Godoy, 2021). This farming method, known as «capture-based aquaculture» (Lovatelli and Holthus, 2008), takes advantage of the species' biphasic life cycle, which includes a planktotrophic larval stage lasting approximately 40–45 days (Toro et al., 2004), which allows for high dispersal potential. Once settled on a suitable substrate, the larvae metamorphose into their adult, sessile form, attaching via proteinaceous byssal threads, which they can regenerate if detached (Laursen, 1992; Silverman and Roberto, 2010).

Most *M. chilensis* farming operations are concentrated in the Chilean northern Patagonia (41.5°S–43°S). The region is characterized by highly

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heterogeneous environmental conditions even at a fine geographic scale (Flores et al., 2022; Curra-Sánchez et al., 2022; González et al., 2010; García-Tuñon et al., 2024). The semi-enclosed inner sea of Chiloé, located in northern Patagonia, features a complex geomorphology with fjords, channels, and islands, divided into four micro-basins: Reloncaví Sound, Reloncaví Fjord, Ancud Gulf, and Corcovado Gulf (Silva et al., 2009). This system is influenced by freshwater input from Patagonian rivers originating on large glaciers on the eastern Andes and oceanic water exchange via the Chacao Channel and Boca del Guafo (Sievers, 2008; Narváez et al., 2019; Vásquez et al., 2021). These geographic and oceanographic conditions generate strong horizontal and vertical gradients in oceanographic variables such as temperature and salinity (Saldías et al., 2021; Vásquez et al., 2021). A stratified circulation pattern is particularly pronounced inside fjords, where oceanographic differences can be observed even within the same locality across different depths (Yevenes et al., 2019; Sievers, 2008; Cáceres and Valle-Levinson, 2010).

In marine invertebrates, dispersal potential—facilitated mainly by planktonic larvae—plays a key role in shaping spatial patterns of genetic diversity (Cowen and Sponaugle, 2009). Species with higher dispersal potential generally exhibit increased gene flow, leading to greater connectivity and reduced genetic differentiation among populations (Kinlan and Gaines, 2003; Haye et al., 2014). Since *M. chilensis* possesses a planktonic larval stage with extended pelagic duration (Toro et al., 2004), high gene flow among northern Patagonian populations promotes genetic connectivity across local populations (Haye and Segovia, 2023; Segovia et al., 2024). Despite the high connectivity pattern, species with moderate and high dispersal capacity can exhibit adaptive differentiation in response to environmental selection pressures, particularly in complex environmental mosaics (Palumbi, 2004; Levin, 2006; Sanford and Kelly, 2011; Segovia et al., 2020). In such cases, differentiation persists due to strong post-settlement selection, leading to the selective mortality of migrants whose phenotypes do not match local conditions (Marshall et al., 2010). The heterogeneous environmental conditions in northern Patagonia can therefore act as distinct local selective pressures, potentially shaping genetic differentiation in *M. chilensis* at a fine geographic scale, as observed in other bivalve species inhabiting heterogeneous environments (Lehnert et al., 2019; Ropp et al., 2023). Fine-scale adaptation can range from a few meters

Table 1
Information on the populations of *Mytilus* spp. from Reloncaví System used in this study. N: number of sequenced individuals.

Source	Acronym	N	Coordinates
Chaparano Wild	CH_W	23	41.62°S; 73.08°W
Chaparano Farmed	CH_F	23	
Bahía Ilque Wild	IL_W	23	41.75°S; 72.57°W
Bahía Ilque Farmed	IL_F	23	

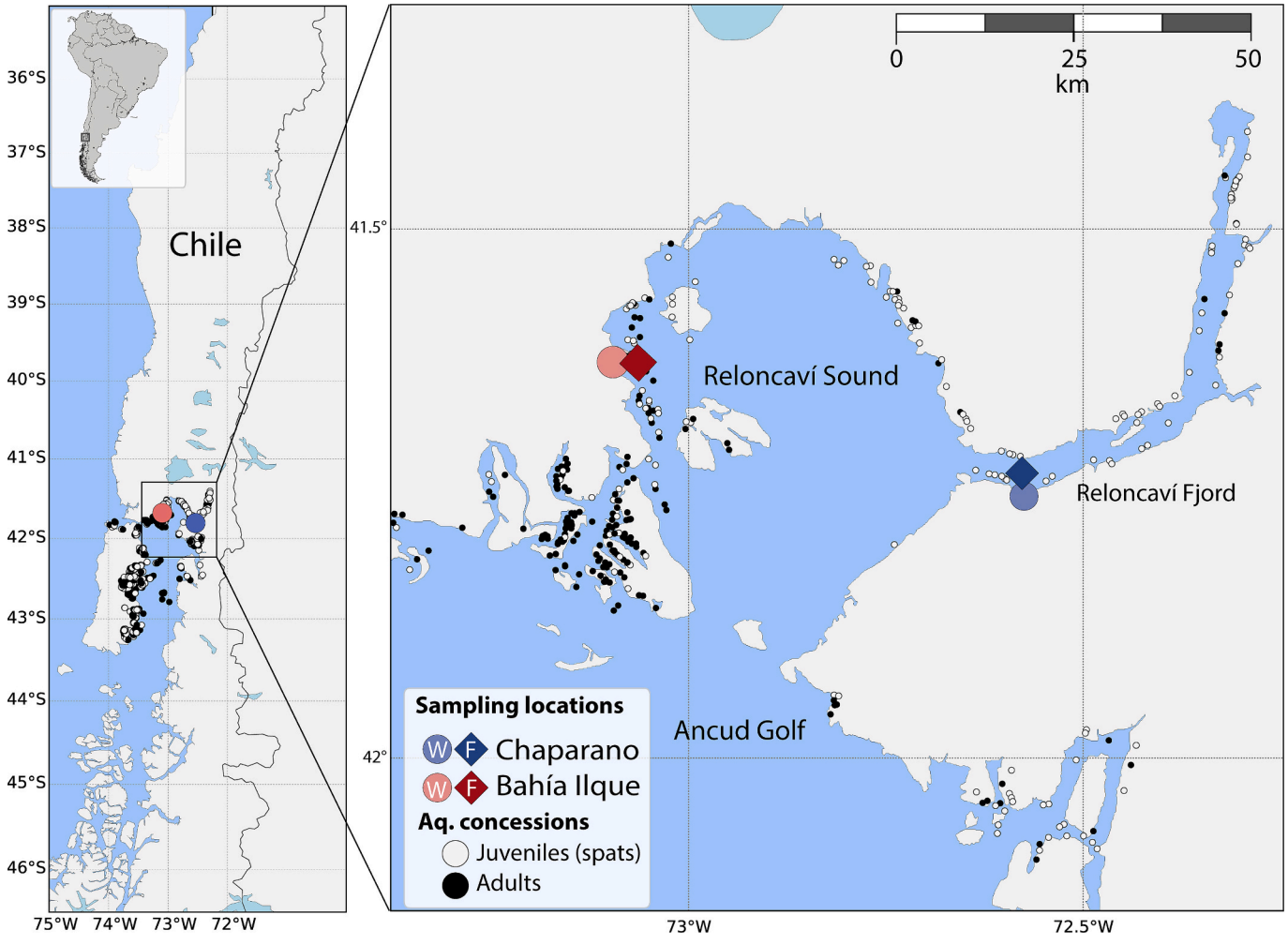

Fig. 1. Map showing the sampling locations of *Mytilus* spp. from both wild (circles) and farmed (diamonds) local populations in the Reloncaví System in northern Patagonia, Chile. It also depicts the distribution of active aquaculture concessions with production records in the Chilean National Fisheries Service (SERNAPESCA), highlighting on-growing centers (dark grey) and juvenile (spat) production sites (light grey). The Reloncaví Fjord area (Chaparano) is marked as one of the key larval production zones.

(Selander and Kaufman, 1975) to hundreds of kilometers (Benestan et al., 2015; Canales-Aguirre et al., 2022), depending on the species' dispersal potential. Here, we define fine-scale adaptation as occurring within a region where populations are highly connected by gene flow, as expected based on their dispersal potential.

To date, molecular studies on *Mytilus* spp. along the south eastern Pacific coast have primarily focused on assessing neutral genetic structure in wild populations using different molecular markers, including allozymes (Cárcamo et al., 2005; Toro et al., 2006), microsatellites (Larraín et al., 2014; Astorga et al., 2020), mitochondrial and nuclear sequences (Astorga et al., 2020; Haye and Segovia, 2023), and SNPs (Araneda et al., 2016; Larraín et al., 2018; del Rio-Lavín et al., 2022). However, in southern Chile, these efforts are further complicated by the occurrence of a *Mytilus* species complex, where *M. chilensis*, *M. galloprovincialis*, and *M. edulis* coexist in sympatry, often leading to hybridization events that blur species boundaries and complicate the interpretation of genetic patterns (Larraín et al., 2018; Larraín et al., 2019; Väinölä and Strelkov, 2011; Jilberto et al., 2024; Oyarzún et al., 2024).

In this context, studies consistently report low genetic differentiation among wild populations, suggesting a panmictic population structure in northern Patagonia, following high genetic connectivity driven by the broad primary and secondary dispersal capabilities of mussel larvae (Wu et al., 2025). Additionally, some evidence suggests interannual genetic variation associated with reproductive success fluctuations (Haye and Segovia, 2023). However, limited research has explored the adaptive genetic structure of *Mytilus* spp. or the influence of environmental factors (i.e., Segovia et al., 2024; Yévenes et al., 2025) on the potential for genetic differentiation between wild and farmed populations.

We hypothesize that despite the high dispersal potential of *Mytilus* spp., which promotes effective gene flow among wild populations, local environmental variability, and habitat conditions of natural and farmed populations, drives adaptive differentiation at a fine geographic scale. This study aims to advance the understanding of genetic patterns in wild and farmed populations by characterizing the population genomic spatial structure using neutral and putatively adaptive loci in a

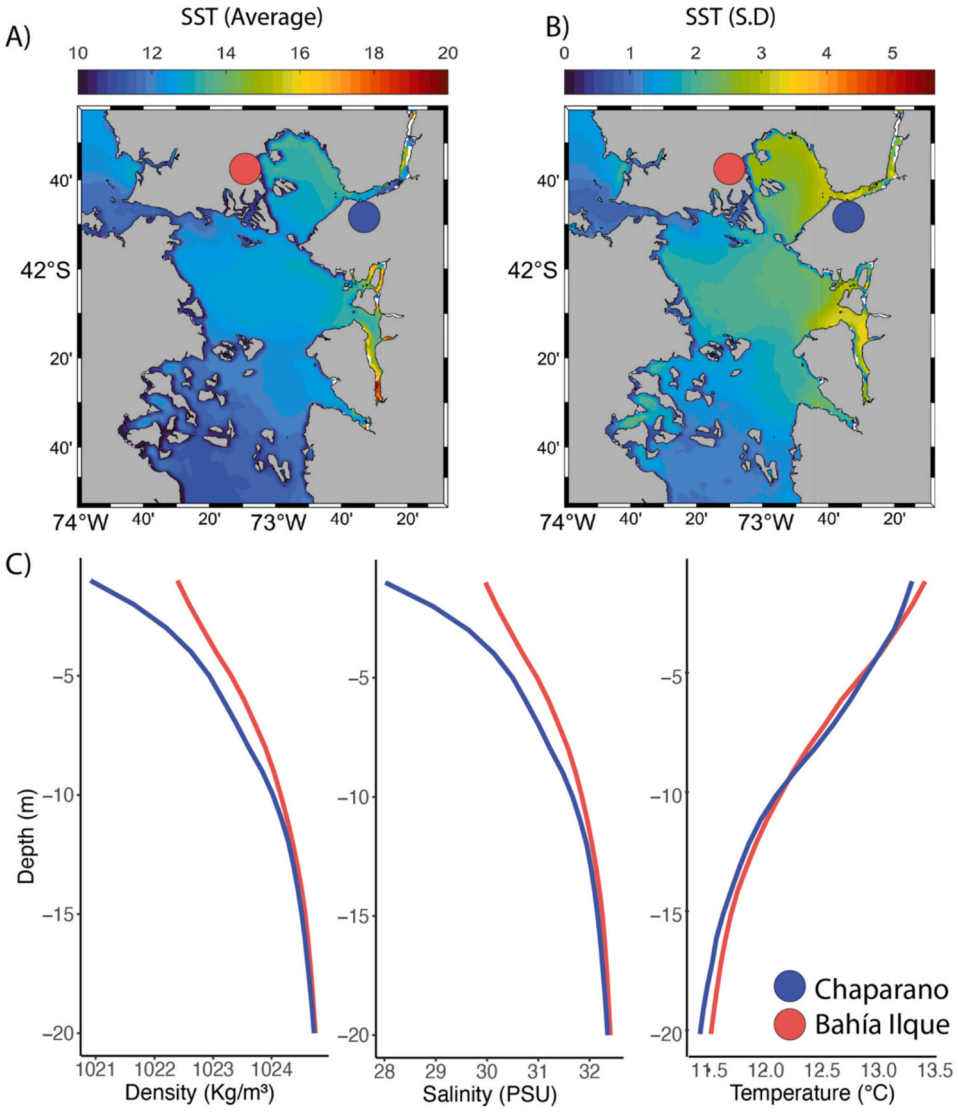

Fig. 2. Environmental patterns of the study area and sites. The maps show (A) the long-term mean sea surface temperature (SST), while (B) represents the standard deviation (S.D.) of SST in the Inner Sea of Chiloé, including the Reloncaví system, on the northwest of the maps (see Fig. 1), based on AQUA-MODIS 1 km resolution data. Additionally, (C) illustrates the long-term vertical variation in density, salinity, and temperature for each of the two sampling areas from monthly in situ measurements collected with a Conductivity, Temperature, Depth sensor (CTD) over nine years (2013−2022) from the oceanographic information system CHONOS initiative of the Instituto de Fomento Pesquero (IFOP) in Chile. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

geographically small but highly environmentally heterogeneous area of northern Patagonia, contributing information for improvement of sustainable management strategies in this region.

2. Methods

2.1. Study area, sampling, and environmental context

This study was conducted in the Reloncaví Sound and Fjord, collectively referred to as the Reloncaví System (RS), located in southern Chile (Fig. 1). Two contrasting sampling sites were selected: Chaparano (41.62°S; 73.08°W), near the fjord's mouth, and Bahía Ilque (41.75°S; 72.57°W), at the westernmost part of the sound (Table 1). The RS exhibits highly dynamic hydrographic conditions driven by the interplay of oceanic waters entering mainly from the southwest, and substantial freshwater discharge from Andean rivers at the fjord's head (Yevenes et al., 2019; León-Muñoz et al., 2013). These interactions, coupled with complex bathymetry, result in strong surface salinity gradients and vertical stratification (Acha et al., 2004) (Fig. 2). Sampling was conducted in December 2022 (austral summer). Wild individuals (CH_W and IL_W) were collected during low tide from intertidal zones within ~50 m of farming areas, with permission from local mussel farmers. Farmed individuals (CH_F and IL_F) were sampled directly from suspended culture ropes at approximately 5 m depth.

2.2. DNA extraction and genotyping-by-sequencing

Adductor muscle tissue was dissected and preserved in absolute ethanol. Genomic DNA was extracted from 25 mg of tissue using the DNeasy Blood and Tissue Kit (QIAGEN, USA), following the manufacturer's instructions. DNA quality and concentration were evaluated using an electrophoresis and Qubit 4 fluorometer (Thermo Fisher Scientific, USA) to ensure purity and integrity. Samples were sequenced using Genotyping-by-Sequencing (GBS) (De Donato et al., 2013) at the University of Wisconsin Biotechnology Center. *ApeK*I enzyme, previously optimized for Segovia et al. (2024), was used for digestion. Unique barcode adapters were ligated to each DNA sample to enable individual identification. Sequencing was performed on an Illumina NovaSeq6000 platform, and quality control was carried out using FastQC (Andrews, 2010).

It is well established that hybridization commonly occurs where multiple *Mytilus* species coexist, complicating species identification (Coustau et al., 1991; Michalek et al., 2016). In southern Chile, this challenge is amplified by the sympatric presence of *M. chilensis, M. galloprovincialis*, and *M. edulis*, together with the limited resolution of single-locus barcoding methods (Larraín et al., 2019; Väinölä and Strelkov, 2011; Jilberto et al., 2024; Oyarzún et al., 2024). Given this context, our analyses focused on the *Mytilus* species complex, recognizing that both natural and farmed populations likely include mixed lineages, with approximately 93.5 % of individuals belonging to a dominant and widespread lineage previously identified as putatively *M.* cf. *chilensis* (Haye and Segovia, 2023). Rather than resolving taxonomy, our aim was to assess fine-scale genetic structure and potential adaptive divergence within a productive system already shaped by this genetic complexity. Accordingly, we retained all high-quality genotypes aligned to the *M. chilensis* reference genome (Gallardo-Escárate et al., 2023; see next section), applying stringent filtering based on mapping and SNP call quality.

2.3. SNP calling and filtering

Raw reads were demultiplexed and filtered using the *process_radtags* module in STACKS 2.6 (Catchen et al., 2013), discarding low-quality reads and retaining only high-confidence sequences. Filtered reads were aligned to the *M. chilensis* reference genome (Gallardo-Escárate et al., 2023) using BWA (Li and Durbin, 2009). SAM/BAM files were processed with Samtools (Danecek et al., 2021) for sorting, indexing, and removal of low-quality alignments or duplicates. SNPs were identified using the *ref_map.pl* pipeline in STACKS. Genotypes were called using the gstacks module, and SNPs were filtered with populations using a minor allele frequency (MAF) threshold of 0.03. Individuals with more than 80 % missing data were excluded, and SNPs with a minimum call rate < 85 % were removed.

Further filtering was performed using Hardy-Weinberg Equilibrium (HWE) deviations, which were estimated using Arlequin 3.5.2.2 (Excoffier and Lischer, 2010) with 100,000 permutations to filter out those loci consistently out of HWE equilibrium in 3 of the 4 sites studied (adding farmed and wild populations). False discovery rate (FDR) correction (Haynes, 2013) was applied (q-value = 0.05), and SNPs in disequilibrium in at least 75 % of sampling sites were discarded to avoid potential sequencing artifacts (Pearman et al., 2022). All the analysis and data filtering were performed using the high-performance computing facilities of the Instituto Milenio en Socio-Ecología Costera (SECOS) Datacenter.

2.4. Outlier detection, genetic structure and gene ontology

Local adaptation putative loci (outlier loci) were identified with BayeScan 2.1 (Foll and Gaggiotti, 2008), using 1,000,000 iterations, a burn-in of 10 %, and a prior odds ratio of 10, and a 5 % False Discovery Rate (FDR) correction to prevent false positives. According to Jeffrey's criterion (Jeffreys, 1961), based on Bayes Factor values (BF > 10), only loci with strong or very strong evidence of selection were considered as candidates.

Genetic diversity (A_r, H_o, H_e, F_{IS}) was calculated for each population using GenoDive 3.05 (Meirmans, 2020). Population differentiation was evaluated using pairwise F_{ST} values in Arlequin 3.5.2.2 with 10,000 permutations. To assess population structure, we used both model-based and multivariate approaches. STRUCTURE 2.3.4 (Pritchard et al., 2000) was run parallelized via Strauto v1.0 (Chhatre and Emerson, 2017) with 500,000 MCMC iterations and 10 % burn-in across ten replicates. The optimal number of clusters (K) was identified using the Evanno method (Evanno et al., 2005) in STRUCTURE HARVESTER (Earl and VonHoldt, 2012) and visualized with Clumpak (Kopelman et al., 2015). Discriminant Analysis of Principal Components (DAPC) was implemented in R using the adegenet 2.1.0 package (Jombart and Collins, 2017). We applied k-means clustering with BIC optimization (*find.clusters*).

Candidate SNPs loci determined with Bayescan were then associated with nearby genes by intersecting SNP coordinates with annotated features from the *M. chilensis* reference genome (Gallardo-Escárate et al., 2023) using BEDTools. Gene functions were inferred through BLASTn searches (https://blast.ncbi.nlm.nih.gov/) based on *E*-value ($1e^{-10}$) and identity thresholds (>70 %).

2.5. Environmental characterization, genomic–environmental associations

We characterized the two sites using vertical profiles of temperature, salinity, and derived water density, based on monthly CTD casts from the IFOP CHONOS program between 2013 and 2022. These measurements extended from the surface to 20 m depth and were obtained through public data requests. Density was calculated from salinity and pressure (Table S2). Although other relevant variables such as food availability (Connor et al., 2016), carbonate chemistry (Navarro et al., 2013; Jahnsen-Guzmán et al., 2021), and dissolved oxygen (Ouillon et al., 2021) have been identified as important, they were not available in this dataset. Satellite-based products provided horizontal surface gradients (Fig. 2) but lacked depth resolution to compare intertidal and mid-water conditions.

To evaluate genotype–environment relationships, we performed a partial redundancy analysis (p-RDA) using the vegan package (Oksanen et al., 2014) in R (R Core Team, 2023). To capture vertical

environmental differences, we used average values from 0 to 3 m for wild samples and 4–6 m for farmed samples. Environmental variables were standardized (*z-score*), and genotypic data were Hellinger-transformed using the *decostand* function in vegan. Model selection was conducted with *ordistep*, comparing a full model with all variables and a null model with intercept only, using 50 steps and 10,000 permutations. In the final model, depth was included as a conditional variable to control for its confounding effect. We performed ANOVA to assess the significance of selected predictors in explaining genetic variation.

3. Results

3.1. SNPs detection

Following initial quality filtering, we obtained a total of 97,750 SNPs aligned with *M. chilensis* reference genome from 91 individuals collected in Bahía Ilque and Chaparano wild (CH_W and IL_W) and farmed populations (CH_F and IL_F). One individual was filtered out due to failure to achieve minimum quality standards. After estimating HWE per locus, 28 loci were excluded as they were consistently found to deviate in at least three of the four populations, resulting in a final dataset of 97,722 SNPs. Using outlier detection with Bayescan, we identified 18 outlier SNPs -representing 0.018 % of the total filtered loci- with strong evidence of being under natural selection according to Jeffrey's criterion (Fig. S1). No individual showed extreme allele frequency patterns deviating beyond what would be expected by chance. This suggests that the observed differences were more likely to reflect population-level differentiation within a shared genetic background, rather than deep divergence associated with the presence of distinct evolutionary lineages.

Of the 18 outlier SNPs identified, four were significantly associated with annotated genes involved in stress response, immune function, or structural defense, specifically, WD40 repeat, mucin, serine-rich adhesin, and fibrinogen-like domain proteins (Table S1). The remaining SNPs either showed no significant gene matches or mapped to unannotated regions.

3.2. Genetic diversity

In the neutral dataset, the effective number of alleles was similar across all populations (1.239–1.247), as were the observed and expected heterozygosity values (0.145–0.150; 0.171–0.176, respectively), indicating a homogeneous distribution of genetic diversity among local populations. The pattern was consistent with F_{IS} values, which were similar among populations (0.142–0.151). In the putatively adaptive (outlier) dataset, the effective number of alleles varied slightly between populations without a clear pattern (1.310–1.575). The highest observed and expected heterozygosity values were found in CH_F (0.237; 0.342, respectively), followed by CH_W (0.194; 0.259) and IL_F (0.159; 0.199). IL_W had the lowest observed heterozygosity (0.145). Additionally, IL_W exhibited the highest F_{IS} value (0.476) compared to the rest of the populations (0.200–0.305) (Table 2).

3.3. Genetic differentiation

Genetic differentiation values estimated using F_{ST} for the neutral dataset were overall low and not significant (Fig. 3). The highest values report slight and non-significant differentiation between the IL_F and all other populations, as well as between CH_W and both IL populations. In the outlier dataset, as expected, F_{ST} values were higher, and significant differentiation was detected between CH_F and its corresponding wild population (0.035), as well as between CH_F and the IL_W (0.125) and IL_F (0.274) (Fig. 3).

Using the method of Evanno et al. (2005), the optimal number of genetic groups was $K = 2$ for both the neutral and outlier datasets (Fig. S2). However, the STRUCTURE analysis for the neutral dataset did not reveal a clear geographic pattern of genetic structuring, except for a subtle differentiation observed in IL_F, consistent with the F_{ST} results. The IL_F population exhibited a lower proportion of ancestry from the most frequent genetic group (Fig. 4) at $K = 2$ compared to the other populations. In contrast, the outlier dataset showed clear differentiation in CH_F, with a higher assignment to the most abundant and spatially widespread genetic group with some individuals assigned entirely (100 %) to this group (Fig. 4).

The Discriminant Analysis of Principal Components (DAPC) did not detect significant clustering for the neutral dataset. However, using as prior the population information of origin and discriminant axes, we observed differentiation along Axis 1 (46.03 %) between the IL_F and both CH sampling sites. Axis 2 (28.82 %) showed a weaker differentiation between IL and CH. In the outlier dataset, four genetic groups were identified based on the BIC criterion; CH_F showed clear differentiation from the other populations along Axis 1 (56.24 %), while CH_W and IL differentiated along Axis 2 (27.59 %) (Fig. 4).

3.4. Environmental characterization and genotype environment association

The environmental characterization of the water column at Chaparano and Bahía Ilque revealed differences in salinity and density. Bahía Ilque exhibited higher salinity levels and weaker stratification compared to Chaparano. Temperature variations between sites were not

Table 2
Genetic diversity of 97,704 neutral SNPs and 18 outlier SNPs of *Mytilus* spp. from Reloncaví System. A_r: Effective number of alleles; H_o Observed heterozygosity; H_e: Expected heterozygosity; and F_{IS}: Inbreeding coefficient.

Dataset	Site	A_r	H_o	H_e	F_{IS}
Neutral	CH_W	1.246	0.150	0.175	0.146
	CH_F	1.239	0.145	0.171	0.151
	IL_W	1.243	0.148	0.173	0.142
	IL_F	1.247	0149	0.176	0.150
Outlier	CH_W	1.435	0194	0.259	0.252
	CH_F	1.575	0.237	0.342	0.305
	IL_W	1.470	0.145	0.277	0.476
	IL_F	1.310	0.159	0.199	0.200

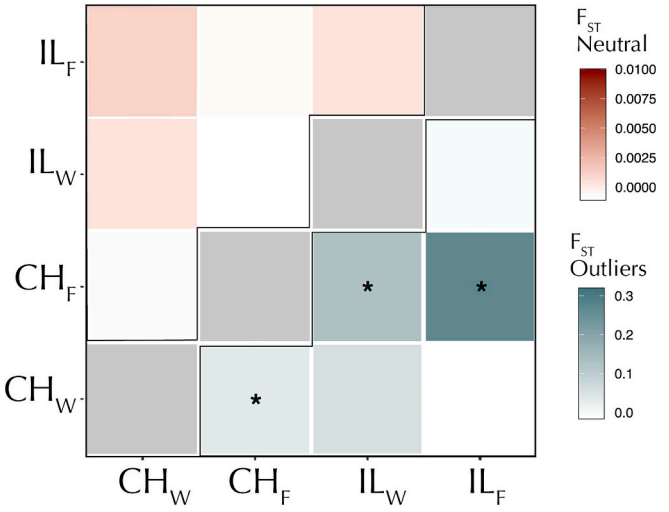

Fig. 3. Heatmap representing pairwise genetic differentiation between *Mytilus* spp. populations from Reloncavi System, based on the F_{ST}. Values above the grey diagonal represent the neutral dataset, and below the diagonal correspond to the outlier dataset (18 SNPs). * Denote significant pairwise values ($p < 0.05$).

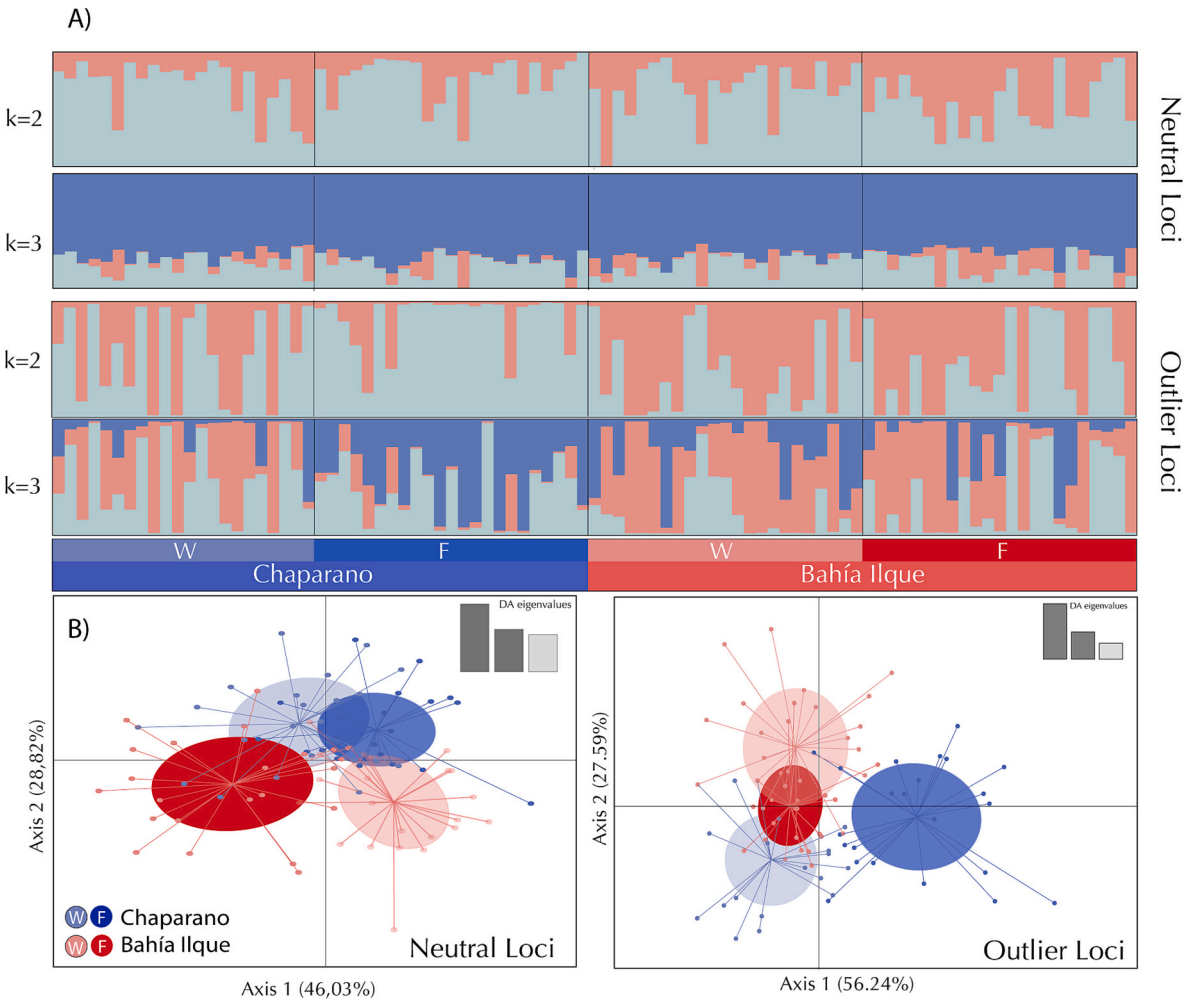

Fig. 4. Genetic clustering of SNPs for neutral and outlier loci of individuals from wild and farmed populations of *Mytilus* spp. in the Reloncaví System, Northern Patagonia. Probabilistic assignment of individuals to detect genetic groups using: Bayesian clustering implemented in STRUCTURE (upper panels), and clustering incorporating the geographic origin of individuals through Discriminant Analysis of Principal Components (DAPC) (lower panels). Dark blue: CH_F; light blue: CH_W; dark red: IL_F; light red: IL_W. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pronounced, but we observed clear differences in the vertical structure (Fig. 2).

We performed a Redundancy Analysis to evaluate the relative influence of the environment on the genetic structure of the putatively adaptive data. The optimal model for the Redundancy Analysis (RDA) in the outlier dataset identified maximum temperature as the most important factor ($F = 5.17$; $p = 0.001$) and salinity range ($F = 2.34$; $p = 0.032$) as key variables influencing genetic structure (Table 3). The overall model was also significant ($F = 3.75$; $p = 0.001$). The first two axes explained 83.61 % of the total variance, and although no strong separation was observed between populations, with a subtle segregation of CH_F, negatively associated with maximum temperature. Additionally, we observed a weaker differentiation between the IL_W and CH_W in association with salinity range (Fig. 5).

Table 3
Summary of the optimized partial Redundancy Analysis (pRDA) model assessing the influence of environmental variables on genetic variation in *Mytilus* spp. from the Reloncaví System. The table shows the degrees of freedom (DF), proportion of variance explained, F-values, and associated *p*-values for each variable included in the final model. Maximum temperature and salinity range were retained as significant predictors of genetic structure, after accounting for depth as a conditional variable.

Variable	DF	Variance	F	p-value
Max. temperature	1	0.0334	5.1731	0.001
Salinity range	1	0.0151	2.3418	0.032
Residuals	87	0.5612		

4. Discussion

Genetic studies on *Mytilus* spp. have traditionally focused on wild populations, given their fundamental role in sustaining the social-ecological system linked to mussel farming and aquaculture (i.e Araneda et al., 2016; Astorga et al., 2020; Haye and Segovia, 2023; Segovia et al., 2024). This study compared farmed and wild populations within the same geographic area, providing the first evidence of a genotypic filtering process in farmed populations in this species even at very small geographic scales (<50 km), suggesting that selective pressures associated with suspended aquaculture could shape the genetic structure of cultivated mussels. This differentiation highlights the potential impact of farming practices on genetic diversity, which may have implications for the sustainability and resilience of the species in the face of environmental variability.

The broader genetic patterns observed in our study are consistent with previous findings for *Mytilus* species, where high larval dispersal potential and anthropogenic seed translocation contribute to maintaining high gene flow and low genetic differentiation among wild populations (Araneda et al., 2016; Astorga et al., 2020; Haye and Segovia,

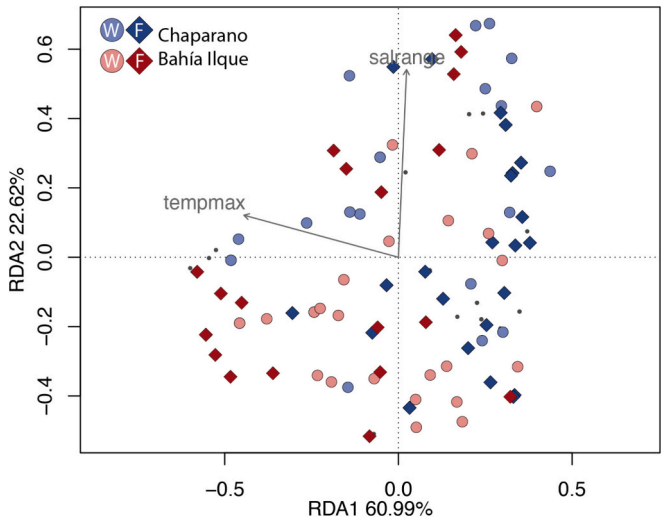

Fig. 5. Optimized Partial Redundancy Analysis (p-RDA) based on 18 outlier SNPs of *Mytilus* spp. in the Reloncaví system, Los Lagos Region, Chile. The axes represent the first two p-RDA dimensions, explaining 60.99 % (RDA1) and 22.62 % (RDA2) of the variance. Vectors indicate the environmental variables that best explain the variation, where tempmax corresponds to maximum temperature and salrange to salinity range.

2023; Segovia et al., 2024). For *Mytilus* spp., substantial effective gene flow has been documented across distant populations, with low genetic differentiation and consistent spatial turnover over time (Haye and Segovia, 2023; Segovia et al., 2024). Thus, the low differentiation observed in our study likely reflects the combined effects of natural dispersal and farming-driven connectivity across the Reloncaví region. We detected genetic homogeneity at the neutral level across all analyzed populations, regardless of whether they were wild or from aquaculture facilities. Importantly, we detected slight genetic differences with adaptive loci between wild and farmed populations, potentially linked to selective pressures. In agreement with previous studies using SNP markers (Araneda et al., 2016; Segovia et al., 2024) and DNA sequences (Larraín et al., 2014; Astorga et al., 2020), the putatively neutral dataset revealed no clear geographic pattern in genetic diversity among populations in the region; with high genetic diversity and no significant differentiation between populations in the Reloncaví System and the inner sea of Chiloé. Similar patterns have been documented in other marine invertebrates with long planktonic larval durations (Cárdenas et al., 2009; Haye et al., 2014; Larraín et al., 2014), supporting the marine connectivity paradigm, which suggests that species with prolonged pelagic larval stages tend to exhibit low levels of population differentiation (Paulay and Meyer, 2006; Selkoe and Toonen, 2011). Nonetheless, this study detected a subtle, albeit non-significant, differentiation in the farmed population of Bahía Ilque compared to the other populations, a pattern that has not been reported previously. The differentiation pattern could be a consequence of the previously reported interannual variation in reproductive success of local wild populations of *M. chilensis* based on temporal variation of the population genetic structure (Haye and Segovia, 2023).

Genetic diversity plays a crucial role for phenotypic plasticity, allowing organisms to cope with environmental variability through morphological and physiological changes (Seebacher et al., 2015; Broitman et al., 2018; Vásquez et al., 2023; Castillo et al., 2024). It has been suggested that in wild populations of *M. chilensis*, phenotypic plasticity in absence of genetic differentiation may the predominant strategy for coping with environmental variability (Haye and Segovia, 2023; Castillo et al., 2024; Segovia et al., 2024). In this study, we provide the first documented evidence of a genetic filtering process in suspended aquaculture populations worldwide, revealing signals of local adaptation primarily in CH_F. For CH_F, there is high confidence in the local origin of farmed individuals, which is supported by its status as a major spat collection area (SERNAPESCA, 2000-2024) and by direct information from local mussel farmers confirming that seeds collected in Chaparano are primarily used for both local farming and for supplying on-growing centers elsewhere (pers. comm.). Importantly, Chaparano does not host commercial on-growing operations, and in consequence does not receive seed translocations from other areas (Fig. 1). Although a few small-scale on-growing centers exist near the head of the fjord, these facilities are limited and rely exclusively on locally produced seeds (Fig. 1). This strengthens the interpretation that the observed genetic differences reflect early farming-driven selective pressures rather than translocation artifacts.

Since our sampling focused on adult individuals, the genetic patterns detected represents the outcome of both random and selective mortality processes at the post-settlement stage, highlighting the potential role of farming conditions in shaping the genotypic composition of surviving individuals. Additionally, a weaker adaptive differentiation was observed between the local populations of Bahía Ilque and Chaparano, regardless of their farmed or wild status. Previous work using SNP markers (Araneda et al., 2016) did not detect differences between wild populations within the Reloncaví System, contrasting with our findings. The closer spatial proximity of sites evaluated by Araneda et al. (2016) likely resulted in less contrasting selective pressures, whereas our broader sampling design, coupled with the use of the newly published *M. chilensis* reference genome (Gallardo-Escárate et al., 2023), enabled a higher-resolution analysis capable of detecting subtle structuring signals.

The RS is a shallow and enclosed basin that exhibits large tidal amplitude (ca. 6 m). Tidal fluctuation is attenuated by the fjord's depth, preventing vertical mixing, and maintaining water mass stratification. The contrasting mixing conditions result in differences in vertical structure between locations in the fjord and sound (Valle-Levinson et al., 2007). Temperature stratification and temperature fluctuate seasonally with maximal (minimal) values during spring-summer (autumn-winter) (Tello and Rodriguez-Benito, 2009; Saldías et al., 2021). Likewise, low (high) surface salinity is observed during the warm (cold) season (Castillo et al., 2016). In this context, the genomic-environmental association analyses suggested that the differentiation between CH_F and CH_W is associated with differences in maximum temperatures and salinity range between the lower intertidal zone and suspended cultures at 5 m depth within the locality (Fig. 2 -Salinity; Jahnsen-Guzmán et al., 2021). Also, adaptive differences between Chaparano and Bahía Ilque appeared to be mainly driven by differences in salinity range. Genetic differentiation at a fine geographic scale has been detected in marine species from estuarine environments, where environmental heterogeneity drives population structuring (Canales-Aguirre et al., 2022; Ropp et al., 2023). Temperature and salinity are key drivers of marine invertebrate distribution (Blanchette et al., 2008; Torres et al., 2025) and have been identified as selective agents promoting adaptive divergence in multiple species (Sanford and Kelly, 2011). Additionally, temperature and salinity can significantly impact growth (Robert et al., 1988; Manoj Nair and Appukuttan, 2003) and survival (Lazo and Pita, 2012; Yuan et al., 2016) of bivalve larvae. In *M. edulis*, temperature modulates filtration rates, which decrease at lower temperatures (Kittner and Riisgård, 2005). Studies on *M. galloprovincialis* have shown that temperature influences adaptive genetic patterns in Mediterranean populations, whereas salinity does not. However, salinity gradients in the Mediterranean are less pronounced than those in the Reloncaví System (Wenne et al., 2022). Furthermore, a reciprocal transplant study of *M. chilensis* in environmentally contrasting locations suggested potential local adaptation of eco-physiological traits associated with salinity (Osores et al., 2017; Yévenes et al., 2025).

The importance of environmental drivers on mussel survival and distributions is also highlighted by recent environmental niche modelling results showing that salinity and temperature are key predictors of future change in the distribution of cultivated mussel species worldwide

(Torres et al., 2025). Other selective pressures beyond environmental drivers may be acting as selective pressures in the farmed populations. As planktonic individuals settle at very high densities onto larval collectors, density-dependent effects may be driving differential mortality patterns that are later compounded by environmental differences among locations where collectors are deployed. To this end, future studies should focus on the differences between the genetic structure of newly settled individuals and that of the remaining individuals after prolonged exposure to grow-out conditions away from the collection sites.

Other studies on mussel aquaculture systems have reported that farmed populations may subsidize wild populations (Norrie et al., 2020; Haye and Segovia, 2023), that artificial structures used for aquaculture can act as stepping-stones enhancing connectivity (Coolen et al., 2020), and that anthropogenic mixing via translocation of individuals facilitates gene flow between wild and farmed populations (Simon et al., 2020). These findings are consistent with the neutral genetic structure results of this study.

Our genomic scans revealed significant differentiation for several outlier loci, chiefly in the farmed population inhabiting the more heterogeneous environment (Chaparano). The pattern suggests a subtle local adaptation process potentially linked to environmental (temperature and salinity) and farming factors (denso-dependency). Among the 18 outliers detected, four were significantly associated with proteins potentially relevant to aquaculture conditions: WD40 repeat proteins, mucins, serine-rich adhesins, and fibrinogen-like domain proteins (Table S1). Proteins containing WD40 domains have been implicated in cellular stress responses and proteolytic regulation, especially under heat and starvation conditions in *Mytilus galloprovincialis* (Venier et al., 2011; Franklin and Gleason, 2025; Mohamed et al., 2014). Similarly, mucin genes are downregulated under low salinity stress in *Crassostrea* spp., suggesting altered oxidative metabolism in hyposmotic environments in mollusks (Schwaner et al., 2023; Yan et al., 2017). Fibrinogen-like domain proteins, commonly involved in innate immune recognition via lectin pathways, have also been found to be differentially regulated under environmental stress in *Mytilus* species (Prego-Faraldo et al., 2018; Zhao et al., 2020). Furthermore, serine-rich adhesins in *Mytilus* likely contribute to byssus formation (adhesion), epithelial integrity, and environmental stress resistance, particularly against salinity, temperature, and mechanical fluctuations (Silverman and Roberto, 2007; Li et al., 2015; Wang et al., 2023). Altogether, these putatively adaptive responses may reflect enhanced requirements for cellular homeostasis, immune surveillance, structural defense mechanisms, which together may point towards density-dependent effects in farmed environments, when compared to wild populations.

The insights from population genetic studies are fundamental for developing management strategies that promote genetic diversity conservation, ensuring sustainable management of the social-ecological system associated with *M. chilensis* farming and mussel cultivation in general. Our results also underscore the importance of considering both natural and anthropogenic factors when studying the genetic structure of *M. chilensis* populations, for example the depth and the existence of vertical environmental refugia (Yevenes et al., 2019; Jahnsen-Guzmán et al., 2021). Further studies are needed to better understand the mechanisms underlying this differentiation, including continuous monitoring of adaptive genetic diversity to evaluate whether temporal genetic variation influences differentiation over time. Additionally, expanding the number of study locations will help determine whether this differentiation is exclusive to Chaparano or occurs in other areas with similar environmental conditions.

5. Conclusions

The spatial patterns of genetic diversity in wild and farmed populations of *M. chilensis* appear to be shaped by high natural and anthropogenic gene flow, which maintains population homogeneity. However, this is the first study to provide evidence of spatially adaptive genetic differentiation in suspended aquaculture populations of *Mytilus* mussels. Differences between populations appear to be driven by contrasting environmental conditions in the fjord and inlet of Reloncaví, particularly maximum temperatures and salinity ranges. The environmental differences are a result from the large continental freshwater inflows into the Reloncaví fjord, creating a dynamic, stratified environment with wide salinity fluctuations and variability even at different depths within a single location.

Our study offerss new insights into the genetic dynamics of this social-ecological system and the influence of environmental variability on its evolution. Importantly, current aquaculture management strategies in southern Chile primarily focus on controlling spat collection and translocation practices. Our findings suggest that farming environments themselves may impose early selective filters on mussel populations, potentially shaping adaptive trajectories even before broader environmental differentiation becomes detectable.

Recognizing this process highlights the need to complement existing management strategies by considering how farming practices influence genetic diversity and population resilience over time. Given the temporal fluctuations in genetic spatial patterns due to differential spat availability in wild populations, ongoing genetic monitoring of both wild and farmed populations can provide major insight into the drivers of adaptation. Expanding the geographic scope of future studies will further enhance our understanding of these dynamics, supporting the development of management strategies that preserve genetic diversity and ensure the long-term sustainability of the mussel aquaculture industry.

CRediT authorship contribution statement

Charel González-Salinas: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Bernardo R. Broitman:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Pilar A. Haye:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Nicolás I. Segovia:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2025.742817.

Data availability

Data will be made available on request.

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