Temporal seascape genomics identifies evolutionary significant units in a highly exploited marine resource, the wedge clam *Donax trunculus*.

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Abstract (194 words)

Clam fishery stocks have experienced a significant, albeit uneven, decline along the Spanish coast, with stock delineation frequently mirroring political boundaries rather than biological ones. Nevertheless, it is crucial to align management units with the biological realities of the species to ensure sustainable exploitation. In this context, understanding the spatio-temporal population structure, genetic diversity, and effective population size (*Ne*) of the wedge clam *Donax trunculus* populations along the Iberian Peninsula coast is vital for making informed management decisions. Thus far, high-resolution genomic data on these key parameters remain limited for this species. Our analysis utilised a substantial dataset of 8,479 single-nucleotide polymorphisms (SNPs) derived from 331 individuals collected over approximately 2,000 kilometres along the Iberian coast, confirming a genetic structure that classified the individuals into three distinct groups: the Atlantic Ocean, the Balearic Sea, and the Alboran Sea. For the first time, we revealed contrasting temporal changes in neutral and adaptive genetic diversity across different fishing areas, reflecting the varying effectiveness of certain management strategies implemented to date. Furthermore, to safeguard genetic diversity and preserve potential local adaptations, we recommend that these genetically distinct groups be managed separately in future conservation, potential translocation and fishing plans.

Introduction

The global fisheries industry holds considerable socioeconomic importance. However, the overexploitation of commercially valuable species, coupled with the impact of global warming, has become a major driver of marine biodiversity decline worldwide (Christian, 2023; Jaureguiberry *et al*, 2022). Economically important bivalves, such as clams, cockles, and scallops, are particularly susceptible to these pressures (Azmi *et al*, 2022; McLaverty *et al*, 2020). Declines in populations often indicate reductions in census size, which can have serious repercussions for genetic diversity, potentially compromising species' resilience and adaptive capacity in the face of environmental change (Thomson *et al*, 2021). Numerous studies have demonstrated that commercial harvesting can significantly diminish the effective population size (*Ne*), consequently accelerating the loss of genetic diversity (Petit-Marty *et al*, 2022). Moreover, the removal of individuals due to fishing can disrupt population connectivity and hinder gene flow (Sadler *et al*, 2023).

The rapid pace of global environmental change highlights the necessity of understanding how human-induced pressures affect populations, species, and communities in order to develop effective conservation strategies for long-term preservation (Kinnison and Hairston, 2007). Advances in molecular techniques have resulted in a growing body of research investigating organismal responses to anthropogenic change, including genomic signatures of adaptation, population connectivity, and genetic diversity (Leigh *et al*, 2019). Although contemporary genetic data can provide valuable insights into current population dynamics, making inferences about historical events based solely on this information can be challenging. The absence of historical baselines complicates the evaluation of changes in effective population size, connectivity, and adaptive potential over time. Temporal genomics, which utilises time-series genetic data from museum specimens or repeated field collections, presents a comprehensive method for addressing these limitations (Snead and Clark, 2022). This approach enables the direct tracking of shifts in allele frequency, assists in disentangling the effects of genetic drift and selection, and links genetic changes to environmental alterations. Such research is crucial for understanding evolutionary responses to anthropogenic pressures and for guiding conservation planning (Clark *et al*, 2023).

A clear understanding of genetic structure, gene flow, local adaptation, and accurate estimates of effective population size is essential for sustainable fisheries management (Benestan, 2020). Fisheries management should be tailored to the local ecological and biological characteristics of each stock, since assuming uniformity or total distinctiveness

without sufficient evidence can produce ineffective or even harmful outcomes (Cadrin *et al*, 2010; Punt, 2023). Identifying the factors that drive genetic variation offers crucial insights into populations' vulnerabilities and facilitates more targeted and adaptive conservation efforts. This approach improves the effectiveness of conservation strategies, ensuring the long-term viability of fisheries (Gandra *et al*, 2021). Furthermore, it aids in the protection of vital ecosystem services, such as food provision and economic support, which are essential for the livelihoods of coastal communities that rely on them.

The wedge clam (*Donax trunculus*) is a commercially and culturally important bivalve distributed along the eastern Atlantic coast from Senegal to France, as well as in the Mediterranean and Black Sea (Baeta *et al*, 2023; Delgado and Silva, 2018; Ramón, 1993). Within the Iberian Peninsula (Portugal and Spain) and across other southern European and North African coastlines, it supports small-scale fisheries and contributes to local economies. As an infaunal filter feeder inhabiting shallow littoral zones across diverse geographical gradients, *D. trunculus* provides a valuable model for studying local adaptation. This species typically resides in fine sandy substrates at depths ranging from 0 to 3 meters in the Mediterranean and Black Sea, and from 0 to 6 meters along the Atlantic coast (Gaspar *et al*, 2002; La Valle *et al*, 2011; Salas-Casanova, 1987).

Despite its ecological role and commercial value, *D. trunculus* has experienced substantial population declines across much of its range, primarily due to sustained fishing pressure compounded by environmental and anthropogenic stressors (Baeta *et al*, 2023; Delgado and Silva, 2018; Reyes-Martínez *et al*, 2015). The Spanish Mediterranean exemplifies this collapse, with official landings decreasing from 1,181.22 tonnes in 1994 to merely 37.04 tonnes in 2024 (Baeta *unpublished data*). This sharp decline has prompted the closure of major fishing grounds, for instance in the Gulf of Valencia (Escrivá *et al*, 2021) and the northern sector of Catalonia in 2020, alongside the functional abandonment of other zones such as central Catalonia and the Almeria coast. A comparable negative trend is observed in Galicia, where landings have steadily declined since the early 2000s, falling below 2 tonnes in recent years (https://www.pescadegalicia.gal/), without signs of recovery. In contrast, the Atlantic coast of Andalusia and Portuguese waters show indications of stock stabilization following earlier declines (particularly during the early 2000s) (https://www.juntadeandalucia.es/; Gaspar personal communication) when landings in both regions dropped considerably. Reported catches have fluctuated between 200 and 400 tonnes annually during the period 2016-2023, suggesting localized recovery or resilience. These regionally divergent trajectories (marked by

collapse in the Mediterranean Sea and Galicia, and relative stabilization in other parts of the Atlantic coast of the Iberian Peninsula) underscore the need for spatially differentiated management strategies that are ecologically grounded and tailored to the population structure and exploitation dynamics of *D. trunculus*.

Current conservation initiatives increasingly rely on genetic assessments to monitor natural populations, as exploitation can negatively impact fertility, larval viability, and growth, thereby heightening extinction risks (Marie et al, 2016; Nantón et al, 2017). Previous population genetic studies on the wedge clam have employed neutral markers such as microsatellites and mtDNA (Fernández-Pérez *et al*, 2018; Marie *et al*, 2016; Nantón *et al*, 2017). For example, Marie *et al* (2016), genotyped in 514 samples from seven localities using 16 microsatellite loci. They identified a clear genetic break at the Atlantic-Mediterranean transition, consistent with the well-documented barriers of the Strait of Gibraltar and the Almeria-Oran Front. These fronts have been shown to limit gene flow in numerous marine species, delineating major biogeographic regions: the Atlantic Ocean, the Alboran Sea, and the north-western Mediterranean (Balearic Sea, hereafter) (Carreras-Carbonell *et al*, 2007; Galarza *et al*, 2009; Patarnello *et al*, 2007; Tintore *et al*, 1988). Accordingly, Marie *et al* (2016) recommended managing these regions separately. This genetic subdivision was further supported by Nantón *et al* (2017) who analysed a distinct set of 15 microsatellite loci and confirmed the existence of genetic groups corresponding to the Atlantic Ocean, and the Alboran and Balearic Seas. They also reported genetic homogeneity along the Atlantic coast of the Iberian Peninsula from Galicia to the Gulf of Cadiz.

In this study, we aimed to (1) document the population genetic structure and connectivity of *D. trunculus* using putatively neutral and potentially adaptive SNP loci, while estimating effective population sizes (*Ne*) and comparing these findings to previous results based on microsatellite data; (2) assess the temporal and spatial stability of the population structure; (3) investigate changes in genetic diversity along the Iberian Peninsula before and after the collapse of wedge clam fisheries, taking into account both formal fishery closures (e.g., in Catalonia, Valencia, and Galicia) and regions where harvesting was gradually abandoned due to declining profitability, utilising temporal sampling from 2011-2014 (pre-collapse) and 2020-2023 (post-collapse); and (4) define management units that reflect the species' demographic history as well as signals of local adaptation. Crucially, we leveraged a unique collection of historical samples previously analysed by Marie *et al* (2016) and Nantón *et al* (2017), allowing us to conduct genome-wide analyses to evaluate the temporal stability of

genetic patterns observed 8 to 11 years ago - prior to the closure of clam fisheries in Catalonia, Valencia, and Galicia. A core and novel of this study compared to Marie *et al* (2016) and Nantón *et al* (2017) is to utilize seascape genomics to test the hypothesis that populations follow a metapopulation dynamics model with clearly defined evolutionary significant units. Seascape genomics examines how spatially variable environmental factors influence genetic diversity over time and space integrating various scientific disciplines such as geography, oceanography, and ecology (Riginos and Liggins, 2013). This approach allows researchers to analyse the effects of migration, genetic drift, and local adaptation through genomic variation patterns (Liggins et al, 2019).

Methods

Sampling design and filtering steps

The sampling plan covers almost the entire range of the species in Spain. Sample collection procedures are described in Marie *et al* (2016) and Nantón *et al* (2017). Samples were collected from seven locations along the Iberian Peninsula, spanning the Atlantic and Mediterranean coasts of Spain and including one site in Portugal (Setúbal) (Table 1, Figure 1). Temporal replicates were collected in 2020, 2022 and 2023 from the same regions sampled by Marie *et al* (2016) and Nantón *et al* (2017) although not always at the exact locations due to logistical constraints.

A total of 331 *D. trunculus* individuals were genotyped using the DArTseq™ platform. To ensure high-quality SNP data, we applied a series of filtering steps based on quality metrics provided by the DArTseq™ pipeline. SNP filtering was performed in R using the dartR (Gruber et al., 2018) and poppr (Kamvar *et al*, 2014) packages. Loci were retained based on the following criteria: a minimum allele frequency (MAF > 0.05), a missing data rate < 35% per locus and per individual, and a pairwise linkage disequilibrium threshold $R^2 < 0.8$ (see Table S1). To avoid biases in population inference, relatedness estimates were calculated to remove full ($r^2 > 0.4$) or half-sibling ($r^2 > 0.2$) individuals using the R package related (Pew et al., 2015). We also generated a neutral dataset by removing of loci that deviated significantly from Hardy-Weinberg equilibrium (HWE) in 70% of the sampling locations, using the *gl.filter.hwe* function in dartR package. This step was conducted after outlier detection to avoid discarding loci potentially under selection, which often deviate from HWE. This filter strategy resulted in

a high-quality SNP dataset characterised by strong reproducibility and suitability for downstream population genomic analyses.

Outlier detection

Outlier loci were identified using two complementary approaches. The first method employed was pcadapt (Luu *et al*, 2017), which detects loci potentially under selection by analysing genotype data at the individual level. This approach performs a principal component analysis (PCA) to capture major axes of genetic variation and then identifies SNPs that are strongly correlated with these axes, suggesting possible adaptive divergence. For each SNP, a q-value was computed, which corresponds to a p-value adjusted for multiple testing, thereby controlling the false discovery rate associated with the large number of SNPs. SNPs with a q-value < 0.05 were considered outliers, i.e. potentially under selection.

Second, we used OutFLANK, an R package implementing the method developed by Whitlock and Lotterhos (2015). OutFLANK detects loci under selection by estimating the distribution of F_{ST} expected under neutrality. It fits a likelihood model to a trimmed distribution of observed F_{ST} values, deliberately excluding extreme values that may represent loci under selection. From this inferred neutral distribution, q-values were calculated for each locus. Loci with q-values < 0.05 were considered outliers, representing candidates for spatially heterogeneous selection. This significance threshold was consistent with that used in pcadapt analysis.

Population structure at both neutral and outlier loci

Population structure was evaluated using both the neutral and adaptive SNP data sets through two complementary clustering approaches. First, we applied a non-model-based *K*-means clustering algorithm using the *find.cluster* function from the *adegenet* package (Jombart, 2008). This method estimates the optimal number of genetic clusters (*K*) by assessing the Bayesian Information Criterion (BIC) across increasing values of K. Prior to clustering, the data were transformed using Principal Component Analysis (PCA) and clustering was performed without assuming any underlying population genetic model.

Second, we conducted a model-based clustering analysis using sparse non-negative matrix factorisation (sNMF) implemented in the LEA R package (Frichot and François, 2015). This method, analogous to STRUCTURE (Pritchard *et al*, 2000), estimates individual ancestry coefficients and infers population structure assuming Hardy Weinberg equilibrium. The optimal number of clusters was determined using the cross-entropy criterion, which evaluates the

accuracy of genotype imputation based on cross-validation. Lower cross-entropy values indicate better model fit. We tested values of K ranging from one to twelve (i.e., $n + 1$, where n is the number of sampling locations), with 10 iterations and a regularization parameter of $\alpha = 1000$. The value of K with the lowest cross-entropy score was selected as the best estimate of the number of ancestral populations.

We conducted a supervised Discriminant Analysis of Principal Components (DAPC) using the *dapc* function in adegenet R package (Jombart *et al*, 2010), by setting priors (*grp*) as sampling locations. An α-score optimization was used to determine the number of principal components to retain, indicating that the first 21 PCs and three discriminant factors should be included in the analysis.

Based on the population grouping identified through using clustering analyses, we computed analyses of molecular variance (AMOVA) using GENODIVE (Meirmans, 2020). The significance of variance components was tested with 20,000 permutations. Populations were grouped into hierarchical sets reflecting the clustering results. As an initial hypothesis, we tested genetic differentiation among three major regions: the Atlantic Ocean and the Alborean and Balearic Seas. This grouping was informed by the presence of two well-established oceanographic barriers, the Strait of Gibraltar and the Almeria-Oran Front, that are known to limit gene flow in numerous marine species.

Temporal genetic trend

Temporal changes in genetic structure and diversity were assessed using samples collected over multiple years at two Mediterranean and three Atlantic locations. These sites were selected to evaluate the genetic consequences of the collapse of wedge clam fisheries in Spain. This collapse was either the result of formal closures (e.g., in Catalonia, Valencia and Galicia) or the result of the progressive abandonment of harvesting activity due to declining profitability. Genetic diversity metrics and summary statistics were calculated for each sampling location and time point. Using the dartR package, we estimated observed heterozygosity (H_o), inbreeding coefficient (F_{IS}) applying default settings (Gruber *et al*, 2018). Pairwise genetic differentiation was assessed by calculating F_{ST} values using the dartR package between all sampling locations and years, based on Weir and Cockerham (1984) estimator, with 10,000 bootstrap replicates and 95% confidence intervals.

Estimating change in effective size

Contemporary effective population sizes (*Ne*) were estimated spatially for each genetic cluster and sampling year using the linkage disequilibrium method implemented in NeEstimator v2 (Do *et al*, 2014). Analyses were conducted with a MAF threshold of 0.05, and all other parameters were set to default. Confidence intervals were calculated using the biased-corrected parametric method.

Additionally, we applied the temporal method to estimate *Ne* for the five sampling locations with temporal replicates: Delta del Ebro, Doñana, Gandia, Ría de Arousa and Vilarrube. The approach estimates *Ne* from changes in allele frequency observed between two sampling points approximately 5 generations apart (assuming one generation per two years). We used Ne Estimator v2. to implement three estimators: Nei and Tajima, Pollak and Jorde and Ryman. A MAF threshold of 0.05 was applied to reduce random noise and computation load.

Spatial variables

To investigate isolation by distance (IBD), we tested the null hypothesis of no correlation between pairwise genetic differentiation [F_{ST} /1- F_{ST})] and in-water geographic distance (*i.e.* within coastline distances). IBD was tested using *mantel.rtest* function from the ape package, with 1,000 random permutations (Paradis *et al*, 2004). In-water geographic distances were calculated using bathymetry maps imported with the *get.NOAA* function from the marmap R package. Least-cost marine path (i.e., shortest paths in water, avoiding land) were computed using the *trans.mat* and *lc.dist* functions.

To further characterise spatial structure, we performed a distance-based Moran's eigenvector maps (dbMEM) analysis using the *dbmem* function from the adespatial R package (Dray *et al*, 2019). dbMEMs represent a spectral decomposition of the spatial relationships among sampling locations, generating (n - 1) orthogonal eigenfunctions that capture spatial patterns at multiple scales (Dray *et al*, 2006). dbMEM variables were derived from the in-water distance matrix. Finally, we applied the *PCNM* function from the PCNM package, using 1,000 permutations. This function transforms spatial distances into a set of orthogonal variables that are suitable for used in constrained ordination analyses (Legendre and Gallagher, 2001) enabling us to incorporate spatial structure explicitly into downstream statistical models.

Environmental variables

Environmental variables – including sea surface temperature (SST), sea surface salinity (SSS), sea floor temperature (SFT), dissolved inorganic carbon, chlorophyll, nitrate, phosphate, dissolved oxygen, iron, pH, phytoplankton - were retrieved from the Marine Copernicus

database (https://marine.copernicus.eu/). For each variable, we extracted monthly minimum, maximum and mean values per year for the period 2010-2023. This timeframe encompasses environmental variation over at least six generations, based on the generation time of *D. trunculus*, which typically ranges from one to two years, depending on environmental conditions such as temperature, salinity, and primary productivity, as well as geographic location (Ansell and Lagardère, 1980; Guillou, 1980; Lamine *et al*, 2020; Mazé and Laborda, 1988; Ramón, 1993; Ramón *et al*, 1995; Zeichen *et al*, 2002).

To incorporate proxies of anthropogenic pressure, we calculated human population density (PDNE) within a 25 km radius surrounding each sampling location. This was derived using shapefiles downloaded from the Global Multi-Resolution Topography (https://www.gmrt.org/GMRTMapTool/) and population data from the NASA earth observations (https://neo.gsfc.nasa.gov/). Additionally, we estimated the percentage of surface land (LAND) within the same 25 km radius around each site to account for coastal topography, which may influence larval retention. Sites with higher percentage of surrounding land were interpreted as more enclosed systems, potentially exhibiting greater larval retention than more open coastal sites.

Redundancy Analysis

Redundancy analysis (RDA) is an ordination statistical technique that extends linear regression to multivariate response data, in this case, individual genotypes across multiple SNPs. First, we calculated individual Euclidean genomic distances using the *dist* function available in the R package adegenet (Jombart *et al*, 2008). We performed a principal coordinate analysis (PCoA) on the individual Euclidean distances, and we kept the resulting principal component (PC) axis. These values represent the position of individuals in the genomic multivariate space and were used as response variable in the distance-based RDA (hereafter dbRDA) while the explanatory variables included spatial structure, represented by Moran's eigenvector maps (MEMs) and the set of environmental variables (Borcard and Legendre, 2002). The code can be found https://github.com/laurabenestan/db-RDA-and-db-MEM. Correlations among Moran's eigenvector maps (MEMs) and the set of environmental variables were assessed and the final model was defined by removing the less significant and non-correlated variables.

Results

SNP filtering

The initial unfiltered SNP dataset generated by DArT comprised 378 individuals and 125,301 loci. Following quality control filtering to obtain a putatively neutral data set, 47 individuals were removed due to low call rate (<65%). In total, 116,822 loci were discarded based on a minor allele frequency (MAF < 0.05), excess heterozygosity (H_{obs} < 0.5), and missing data thresholds (<65%; Table S1). Additionally, six closely related individuals were identified and removed to avoid bias.

Outlier detection analyses identified loci potentially under selection: 679 loci were flagged by PCAdapt and 306 by OutFLANK (Figure S1), with 280 loci overlapping between both methods. The final dataset retained 325 individuals and included two subsets: 7,774 putatively neutral SNPs and 705 putatively adaptive SNPs. All analyses described hereafter were conducted using both datasets.

Broad scale neutral and adaptive population structure

Using a dataset of 7,774 putatively neutral SNPs, we identified two major spatial genetic clusters along the Spanish coastline (K = 2, CV = 2007), corresponding to the Atlantic and Mediterranean regions, as revealed by both K-means clustering and sNMF analysis (Figure 1, Figure S2).

In contrast, analysis of the 705 putatively adaptive SNPs revealed more refined structure. The sNMF results supported three ancestral groups that corresponded to distinct geographic regions: (i) the Atlantic Ocean, (ii) the Balearic Sea, and (iii) the Alboran Sea (Figure 1). K-means clustering, however, indicated four ancestral groups, further subdividing the Atlantic region into a southern Atlantic cluster, comprising only Doñana samples, and a central Atlantic cluster including Vilarrube (VI), Ría de Arousa (RI), and Setúbal (SE). The difference in model fit between K = 3 (CV=1301), K = 4 (CV = 1296), K = 5 (CV = 1296) was marginal, and no additional spatial structure emerged at K equal or up to 5. Most individuals showed high assignment probabilities (>70%) to their respective cluster, with limited admixed (Figure S2). Notably, individuals from the Doñana (DO) population displayed evidence of genetic admixture between the South and North Atlantic clusters.

This pattern was corroborated by the Discriminant Analysis of Principal Components (DAPC) using outlier SNPs, in which the first axis (82.2%) clearly separated individuals from the Balearic, Atlantic, and Alboran groups, while the second axis (15.5%) distinguished the Alboran and Atlantic groups (Figure 2). The third axis (1.1%) distinguished the North and South

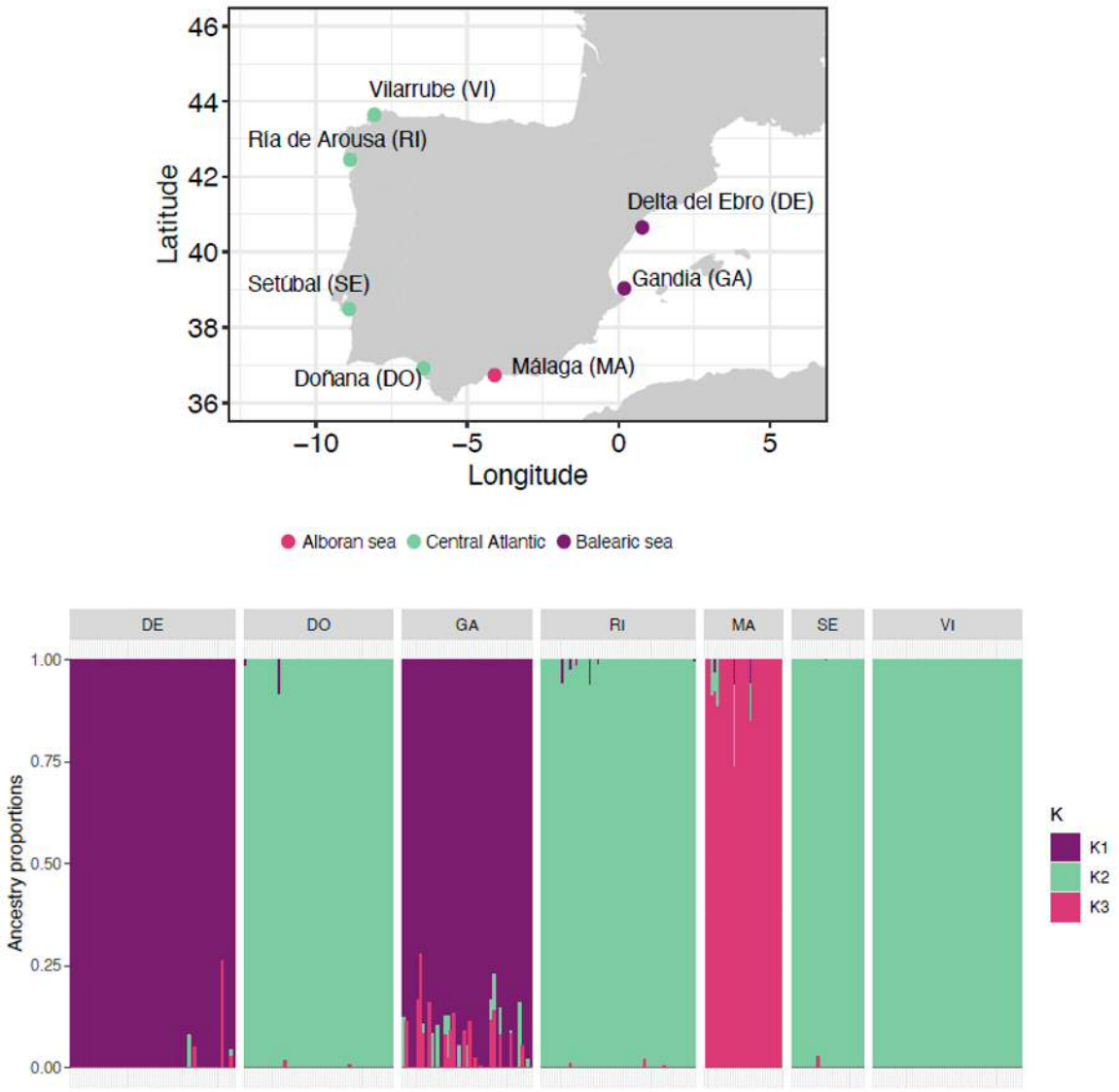

Figure 1. Spatial population structure. (top) Map of the sampling locations in the Iberian Peninsula and (bottom) broad-scale population genomics structure in putatively neutral SNPs. Ancestral probabilities based on the sNMF algorithm for K = 2 using outliers. Genetic clusters are represented by different colours. The colour patterns correspond to regions as follows: green shades = Central Atlantic; purple shades = Balearic Sea; magenta = Alboran Sea. Each bar represents a different individual.

Atlantic groups (Figure 2). Interestingly, individuals from the Alboran Sea clustered slightly closer to the Atlantic group than to the Balearic, reflecting their intermediate geographic position. Atlantic individuals formed a more compact cluster in the DAPC space compared to those from the Balearic, suggesting lower within-region genetic variability. However, when Atlantic and Balearic samples were analysed separately, no additional genetic substructure was detected within either region. Similarly, examination of additional principal coordinate axes did not uncover further structure (results not shown).

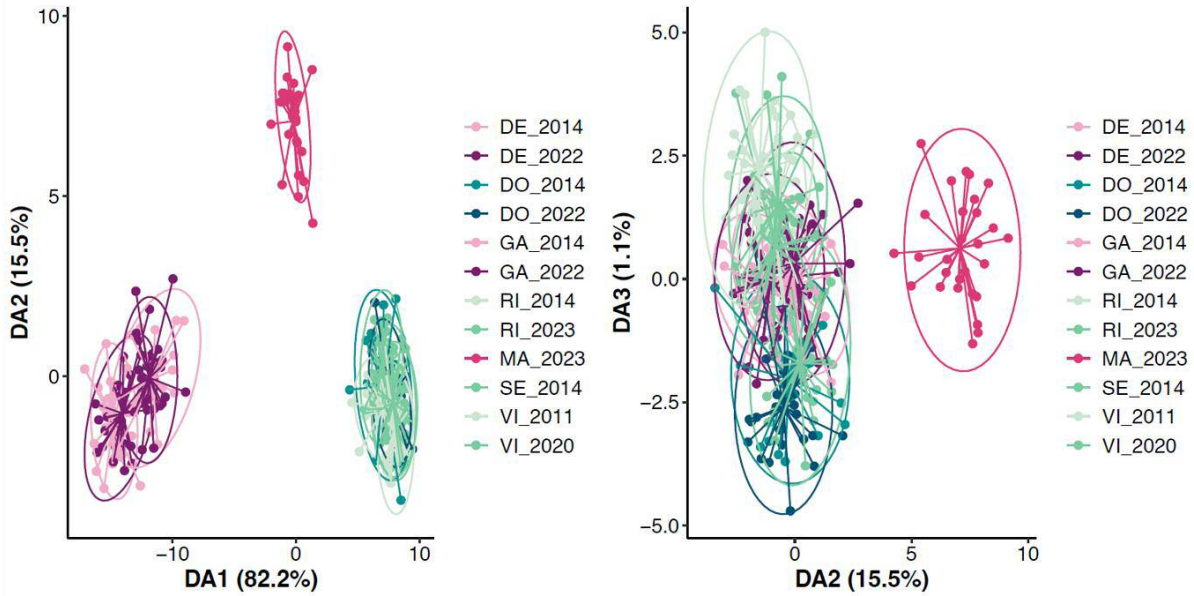

Figure 2. Temporal population structure. Discriminant Analysis of Principal Components (DAPC) across time and sampling locations using 280 outliers. Each point represents an individual, color-coded by sampling site, consistently with the sampling map.

The Analysis of Molecular Variance (AMOVA) on the four genetic clusters found by both K-means and DAPC and conducted on the neutral dataset revealed high within-site variability (F_{IS} = 51.7%, P-value < 0.001), alongside significant but low variation among populations within clusters (F_{SC} = 0.3%, P-value < 0.001). In contrast, variation among clusters was notably higher (F_{CT} = 5.4%, P-value < 0.001; Table 2).

Temporal population structure

The temporal stability of the genetic structure was further supported by the DAPC results, in which temporal replicates from the same location consistently clustered together (Figure 2). Consistently, the lowest and non-significant (P-values > 0.01) pairwise F_{ST} values were found between temporal replicates (Table S2), including DE_2014/DE_2022 (F_{ST} = 0.0013, P-value = 0.02), DO_2014/DO_2022 (F_{ST} = 0.0010, P-value = 0.05), GA_2014/GA_2022 (F_{ST} = -0.0005, P-value = 0.71), RI_2014/RI_2023 (F_{ST} = 0.0015, P-value = 0.01), and VI_2011/VI_2020 (F_{ST} = 0.0011, P-value = 0.06). An exception was observed in the VI/RI comparison, which shifted from a non-significant value before the fishery closure (F_{ST} = 0.0005, P-value = 0.14) to a significant one afterward (F_{ST} = 0.0021, P-value < 0.001). The highest and most significant levels of genetic differentiation were consistently detected between Atlantic and Mediterranean sites, highlighting limited gene flow between these two basins. At a finer spatial scale, all pairwise comparisons revealed statistically

significant genetic differentiation (P-value < 0.001), underscoring the strong genetic singularity of each sampling location - except for comparisons involving temporal replicates and the VI_2011/RI_2014 pair.

Overall, population structure remained stable over time in all locations (except for VI and RI), as evidenced by a strong correlation between pairwise F_{ST} values before and after the fishery closure ($R^2 = 0.8323$; P-value < 0.001). Prior to the closure, pairwise F_{ST} values based on neutral loci ranged from 0.0005 (VI_2011 vs. RI_2014) to 0.0976. Following the closure, the F_{ST} range slightly increased, spanning from 0.0021 to 0.0976. This consistency in genetic differentiation patterns suggests limited temporal fluctuations in gene flow and population connectivity over the study period.

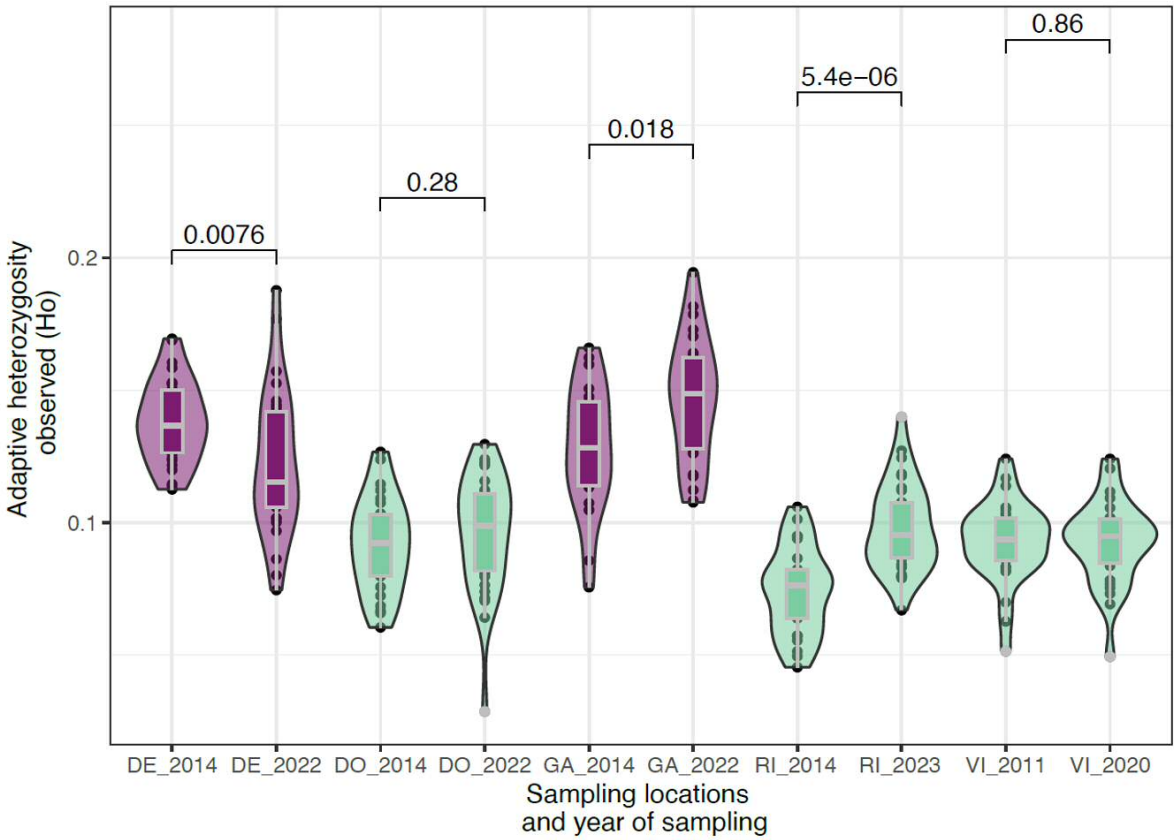

Figure 3. **Genetic diversity changes over time** - Adaptive genetic diversity across the year is shown for the five sampling locations with temporal replicates. Statistical tests were performed between temporal replicates, and corresponding P-values are indicated. Each point represents an individual, with colours corresponding to those used in the sampling map.

Genetic diversity and effective population size across time

Observed heterozygosity across sampling locations ranged from 0.08 to 0.16 and from 0.05 to 0.20 for putatively neutral (Figure S3) and adaptive SNPs (Figure 3), respectively. In two out of five locations with temporal replicates, no significant temporal differences in either

neutral or adaptive genetic diversity were detected, indicating relative stability over time. However, two sites, one in the Balearic Sea (DE) and one in the Atlantic Ocean (RI), exhibited significant (P-values < 0.01) but contrasting temporal trends. Specifically, DE showed higher genetic diversity prior to the fishery closure, while RI exhibited increased genetic diversity following the closure.

At the regional scale, the effective population size (*Ne*) in the Balearic Sea was significantly higher in the most recent temporal replicate (2022), reaching 4,225 (95% CI: 3,366-5,669), compared to 2,861 (95% CI: 2,390–3,564) in the earliest replicate (2014). This indicates an almost twofold increase over time. Conversely, the Atlantic population showed a marked decline in *Ne*, dropping from 1,174 (95% CI: 1,139–1,210) in pre-collapse period (2011 and 2014) to 718 (95% CI: 707–730) in post-collapse period. At a finer spatial scale, contemporary population sizes were low across all locations, ranging from 70.6 (DE) to 100.1 (GA; see Table 3). Notably, the highest estimates of *Ne* were recorded in RI (*Ne* = 93.3, CI = 88.1 – 98.8), which is the location where significant increase in genetic diversity was observed, and in GA (*Ne* = 100.1, CI = 91.9 – 109.5).

Seascape genomics

When examining isolation by distance (IBD) across all Atlantic sampling sites, we identified a significant correlation between genetic and marine distances within the Atlantic cluster ($R^2 = 0.103$; P-value < 0.001). Broad-scale spatial structure also had a significant impact on genetic variation, as demonstrated by the distance-based redundancy analysis (dbRDA) using all samples. This structure accounted for 4.14% of the total genetic variance at neutral loci, while environmental variables contributed only 2.22%.

The environmental predictor variables (PC1, PC2, PC3) exhibited strong correlations with spatial eigenvectors (MEM3, MEM10, MEM11), with all pairwise correlations exceeding 0.7. To address collinearity, we retained those variables that demonstrated the highest explanatory power based on individual linear models. This selection led to the inclusion of five variables in the final dbRDA model: PC1, PC3, MEM4, MEM10, and MEM11. All variance inflation factor (VIF) values were below 5, indicating an acceptable level of collinearity (with VIF > 10 typically regarded as problematic). The final model was statistically significant, with an adjusted R^2 of 4.14%.

The analysis of outlier SNPs revealed that spatial structure accounted for a greater proportion of genetic variation (P-value < 0.001, adjusted $R^2 = 0.18$) than environmental factors

(P-value < 0.001, adjusted $R^2 = 0.10$). Following an assessment of collinearity, we retained the same set of explanatory variables used in the neutral SNPs analysis. The final model for outliers was also significant, displaying a higher adjusted R^2 of 0.178 (Figure 4).

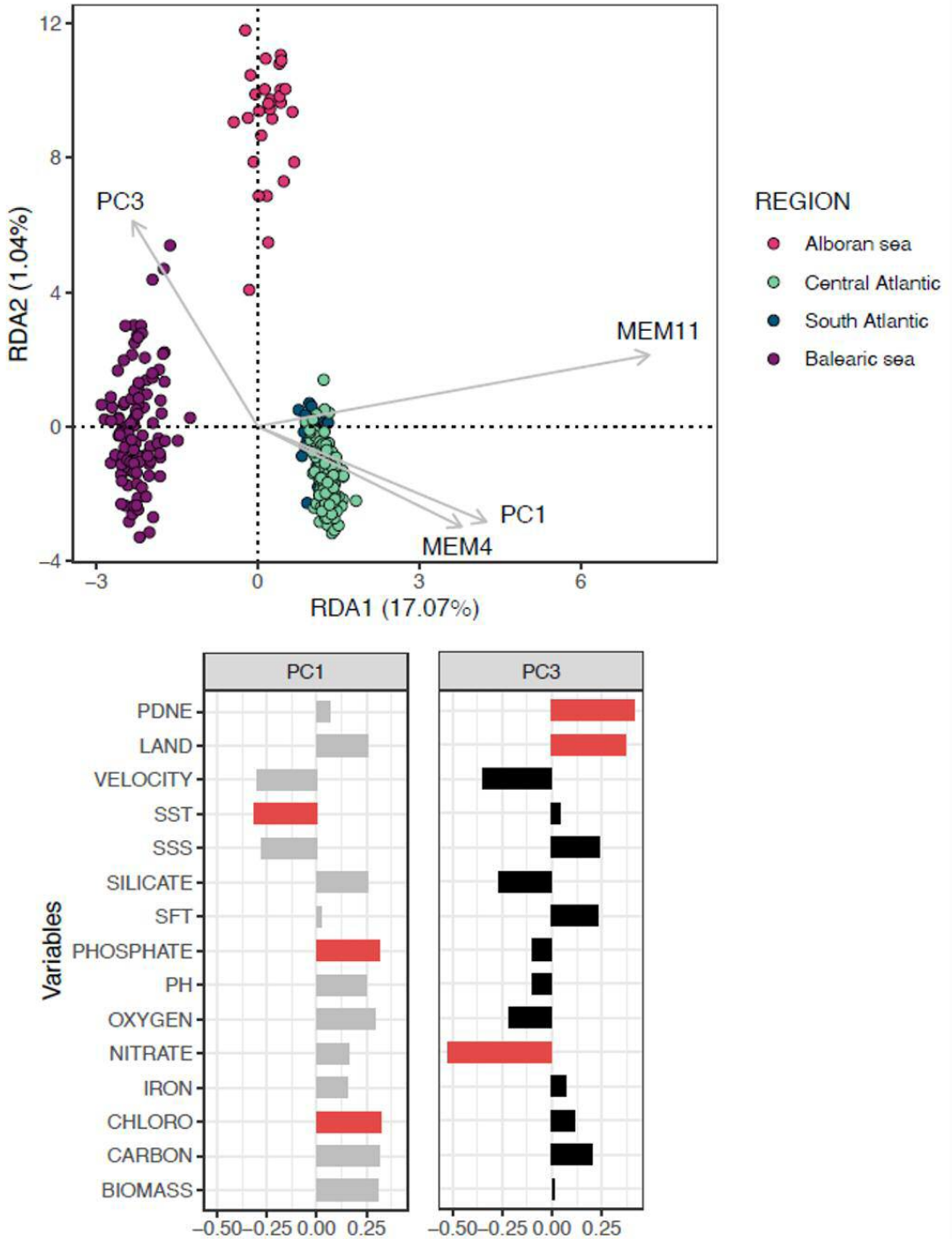

Figure 4. **Genotype by environment**. (a) The influence of spatial, temporal and environmental variables on the adaptive population structure. The location of individuals in the ordination space reflects their relationship with the environmental variables based on their multilocus genotypes at the 705 outliers. (b) The contribution of each environmental variable to the two principal components is shown; the three variables with the highest contributions to each axis are highlighted in dark red.

This model indicated that PC1 values, which are predominantly influenced by phosphate, carbon, and chlorophyll, are higher for the Atlantic region. In contrast, PC3 values, which include PDNE, LAND, and negatively nitrate, are elevated in the Balearic Sea samples.

Discussion

This study represents the first seascape genomics assessment of the commercially important wedge clam, *D. trunculus*, along the Iberian Peninsula coast. We uncovered temporally stable genetic structure marked by distinct spatial discontinuities spanning approximately 2,000 km of coastline. Although overall genetic differentiation was moderate ($F_{ST} < 0.1$), our analyses revealed clear stock boundaries and fine-scale population structuring, refining previous insights derived from microsatellite data (Marie *et al*, 2016; Nantón *et al*, 2017) and offering higher resolution view of the species' spatial genetic organization.

Temporal stability of genetic connectivity

Understanding the evolutionary consequences of anthropogenic pressures on natural populations is vital for assessing their evolutionary trajectories and predicting long-term resilience. In this study, we found no significant changes in population connectivity between samples collected before and after the collapse of most wedge clam fisheries in Spain, which occurred either due to formal closures or the abandonment of harvesting activities stemming from declining profitability. This suggests that genetic drift has had a limited impact on allele frequency changes during the examined time frame. This finding is consistent with (Pinsky *et al*, 2021), who reported temporal genomic stability in Atlantic cod despite decades of exploitation, based on widely spaced temporal replicates (e.g., 1940 and 2013). The observed genetic homogeneity over time implies that individuals within populations display consistent responses to environmental variability across years. A notable exception was the comparison between Ría de Arousa and Vilarrube, where genetic differentiation was not significant in pre-collapse period but became significant in post-collapse period, indicating possible recent changes in connectivity or localised demographic processes.

Sharp isolation between regions

A sharp biogeographic break between the Atlantic Ocean and the Mediterranean Sea has been documented across a wide range of species (Patarnello *et al*, 2007) and more recently confirmed through genomics approaches combined with large scale sampling designs (Boulanger *et al*, 2022). In this study, we confirmed this pronounced genetic break in populations of *D. trunculus*, alongside more subtle signals of divergence within the Atlanto-

Mediterranean transition zone, notably the distinctiveness of the Alboran Sea cluster. In contrast, no genetic structuring was detected within either Mediterranean or North Atlantic populations, suggesting that genetic uniformity in these regions reflect a relatively recent evolutionary history.

Significant ocean barriers in the Atlantic Ocean and the Western Mediterranean Sea, specifically the Gibraltar Strait and the Almeria-Oran Front (AOF) have been identified as effective environmental discontinuities limiting geneflow in *D. trunculus* and other species (Marie et al, 2016; Nantón et al, 2017; Patarnello et al, 2007). Our analysis using potentially adaptive loci confirms that these barriers restrict gene flow, leading to increased genetic differentiation over time, a pattern consistent with observations in other marine species (Galarza *et al*, 2009). This genetic differentiation highlights the important role of oceanographic dynamics in shaping population structure. Before Atlantic waters flow through the Gibraltar Strait, they recirculate near Cape Trafalgar, influenced by strong tidal currents and an underwater ridge (Sala *et al*, 2023). This interaction creates a cold-water patch that acts as a barrier for certain species, restricting their migration between the Atlantic Ocean and Mediterranean Sea. Such oceanographic features can have cascading effects on the ecosystem, impacting the distribution and abundance of various marine organisms (Marie *et al*, 2016; Nantón *et al*, 2017; Patarnello *et al*, 2007). Understanding these dynamics is therefore essential for the development of effective conservation strategies to protect the region's biodiversity.

The AOF, located approximately 400 km east of the Gibraltar Strait, is identified as a semi-permanent dynamic front formed by the confluence of distinct water masses. It serves as a barrier to gene flow for several species (Sánchez-Garrido and Nadal, 2022). Our data reveal significant genetic differences among individuals from the Atlantic Ocean, the Alboran Sea, and the north-western Mediterranean coast, suggesting the AOF's role as a critical boundary for genetic connectivity. The unique environmental conditions and contrasting water properties on either side of the AOF likely contribute to this genetic divergence. Further research into the mechanisms by the AOF influences gene flow could offer clues regarding the evolutionary processes shaping marine biodiversity in this region.

Local adaptation

The use of outlier markers has revolutionised the identification of evolutionary significance units (ESU) by capturing signals of adaptive variation (Benestan, 2020; Funk *et al*, 2012; Gagnaire *et al*, 2015). Our findings offer greater resolution than earlier microsatellite-based studies, which identified three distinct regions, the Atlantic Ocean, the Balearic Sea, and

the Alboran Sea, as separate management units based on genetic differentiation (Marie *et al*, 2016; Nantón *et al*, 2017). Patterns observed at potentially adaptive loci distinctly differentiate populations in the South and Central Iberian Atlantic from those in the Alboran and Balearic Seas. Importantly, our results emphasise the influence of environmental variables, such as carbon concentration, seafloor temperature, and primary productivity (chlorophyll-a), on selecting forces that shape genetic variation in *D. trunculus*. This aligns with previous seascape genomics studies on marine invertebrates, which have recognised ocean temperature and chlorophyll as key drivers of population structure in marine invertebrates (Nielsen *et al*, 2020), and particularly in scallops (Hollenbeck *et al*, 2022; Van Wyngaarden *et al*, 2018). In another clam species (*Ruditapes decussatus*), transcriptomic responses to temperature differ between Atlantic and Mediterranean populations (Saavedra *et al*, 2023), further supporting the role of thermal regimes in adaptive divergence. Comparative studies across 195 bivalve species have also demonstrated the influence of chlorophyll and temperature on growth dynamics (Saulsbury *et al*, 2019). Together, these findings point to region-specific adaptive pressures and underline the importance of implementing conservation strategies tailored to the unique evolutionary trajectories of each population.

Local variability of genetic diversity and population size

The overexploitation of marine species leads not only to population declines but also to disruptions in key ecological processes, such as larval recruitment, which are essential for preserving genetic diversity and ensuring long-term population resilience. Temporal estimates of effective population size (*Ne*) across different regions indicate contrasting evolutionary trajectories: signs of demographic recovery have been observed in the Mediterranean Sea, while a significant decline has been recorded in Atlantic populations. The maintenance of fishery closures in the Atlantic is vital for effective management and restoration strategies, as these findings highlight. By integrating genetic diversity into marine management plans, the long-term health and resilience of these ecosystems can be protected, ultimately benefiting the unique biodiversity of each area (Flanagan *et al*, 2018; Holderegger *et al*, 2006).

Reductions in *Ne* following fishery collapse have been documented in various marine species (Ciannelli *et al*, 2013; Pukk *et al*, 2013) although this pattern is not universal (Cuveliers *et al*, 2011). For instance, some depleted populations still exhibit high genetic diversity, even higher than those in healthy abalone fisheries (Wooldridge *et al*, 2024). Alarmingly, effective population sizes across all sampled *D. trunculus* populations remain below or near the critical threshold of 100 individuals. This is of particular concern, as maintaining an $Ne \geq 100$ is

required to limit inbreeding depression to less than 10% over five generations (Frankham *et al*, 2009). The concept of *Ne* is essential for the survival and evolutionary potential of small populations. According to updated guidelines by Frankham *et al* (2014) the classic 50/500 rule has been revised, recommending a minimum *Ne* of 100 individuals to avoid short-term inbreeding risks and suggesting that populations with *Ne* below 500–1000 are at high risk of long-term extinction due to genetic erosion. Maintaining populations above critical thresholds is essential for conservation, as it enhances their ability to adapt to environmental changes. Due to extensive exploitation of *D. trunculus* across much of its geographic range, this species is at an increased risk of extinction.

Notably, using microsatellites, (Marie *et al*, 2016) reported that the Doñana population was the only sample with the effective population size appeared sufficiently large (*Ne* = 1191, CI low = 526, CI high = infinite) to suggest no immediate risk of extinction. They attributed this finding to the strict fishery management practices in Doñana National Park. Since 1996, the park authority has regulated the *D. trunculus* fishery by restricting harvesting to 160 licensed fishermen. These harvesters must use an artisanal hand-dredge with an iron structure and a large mesh, as boat fishing is prohibited in the intertidal zone. They may collect up to 25 kilogrammes of *D. trunculus* per day, five days a week, and the minimum catch size is 25 mm shell length. Additional restrictions include a two-month annual closure during the reproductive season and bans on harvesting when toxic algae are present (Marie *et al*, 2016). In contrast, using SNPs rather than microsatellites, we found that the Doñana population is in fact at risk of extinction, as all *Ne* estimates fell below 100 individuals (*Ne* Nei Tajima: 80.7, CI low = 75.8, CI high = 86.1; Table 3). SNP-based *Ne* estimates exhibited narrower confidence intervals compared with those obtained from microsatellites. This difference reflects the consensus that SNPs provide more robust estimate of effective population size, owing to their uniform genome-wide coverage, stable and low mutation rates, reduced susceptibility to homoplasy, and higher precision (Olah *et al*, 2024; Zimmerman *et al*, 2020). Given the critically low *Ne* revealed by SNP data, urgent conservation measures are needed to mitigate extinction risks. This should include establishing no-fishing areas, systematic monitoring of environmental health and community-based awareness campaigns to promote sustainable practices and strengthen local engagement in conservation efforts.

Regional comparisons indicate that *Ne* estimates obtained in this study align with or improve upon previous estimates, especially in the Balearic Sea, where a nearly twofold increase was observed between 2014 and 2022 samples. Continued monitoring and the

enforcement of conservation measures will be essential to preserve the long-term evolutionary viability of these populations. At a finer spatial scale, we detected increases in genetic diversity over time in Ría de Arousa (RI), while a decline was observed in the Ebro Delta (DE). These patterns appear to reflect differences in local management practices and fishing pressure. In Ría de Arousa, official records of clam harvesting vary depending on the exact beach where shellfishing activities occur. For example, there are no records for A Lanzada (the site where RI_2014 was collected) for the past 25 years, suggesting a long-term absence of exploitation that may have contributed to the maintenance or recovery of genetic diversity. In contrast, the Ebro Delta has never experienced a complete cessation of fishing activity. Although vessel-based, mechanised dredge fleets targeting larger individuals in deeper waters (0.7–2.5 m) have largely ceased operations, a persistent and intensive fishery remains in the shallow infralittoral zone (< 0.7 m), where on-foot shellfishers using hand-operated dredges continue to harvest smaller clams. This ongoing pressure may partly explain the observed decline in genetic diversity at this site, despite past management interventions.

Translocation plans

The deliberate translocation and release of organisms beyond their historical or current natural range, as part of conservation strategies aimed at establishing new populations of species of concern, commonly referred to as conservation translocations (Griffith *et al*, 1989) remains a topic of considerable debate (Müller and Eriksson, 2013; Ricciardi and Simberloff, 2009). Despite the ongoing controversy, such interventions have become increasingly common in response to the urgent need for pragmatic tools to address the rapid decline of biodiversity. In this context, the uniform genetic structure and stable levels of genetic diversity observed across northern and central Atlantic populations (i.e., Vilarrube, Ría de Arousa, and Setúbal) suggest that translocations between these locations may offer a viable and low-risk strategy for reinforcing depleted populations. Such measures could enhance genetic resilience and improve the long-term adaptive potential of these groups. However, the success of any translocation initiative will depend on careful and ongoing monitoring to assess demographic and genetic outcomes.

Conversely, the partial genetic distinctiveness observed in the Doñana population suggests that researchers exercise caution when considering translocations involving southern Atlantic stocks. In the Balearic Sea, the lack of genetic substructure between populations from Gandia and the Ebro Delta indicates high levels of connectivity and overall homogeneity at

neutral loci. This offers promising opportunities for coordinated management actions, assuming translocations remain within genetically cohesive units. Nevertheless, these findings also demonstrate the need for comprehensive genomic assessments to ensure that any intervention is ecologically and genetically sound. As in the northern Atlantic, translocations between genetically similar Mediterranean populations may offer a low-risk option for population reinforcement, provided local adaptive variation is respected. In contrast, populations from the Alboran Sea (*e.g.*, Malaga) show clear genetic differentiation, particularly at loci potentially under selection. This pattern supports treating the Alboran Sea as a distinct management unit. As such, we should avoid translocations across known genetic barriers, such as the Almeria-Oran Front, to preserve the integrity of locally adapted gene pools. Maintaining these distinct evolutionary lineages is essential for safeguarding ecosystem function and species resilience under changing environmental conditions.

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Table 1. Sampling locations with their corresponding site code, year of sampling, geographic coordinates and number of samples successfully genotyped

Sampling locations	Code	Latitude	Longitude	Year of sampling	n
Vilarrube	VI	43.64250000	-8.07500000	2011	29
				2020	27
Ría de Arousa	RI	42.44750000	-8.87555556	2014	25
				2023	31
Setúbal	SE	38.48222222	-8.90416667	2014	28
Doñana	DO	36.91222222	-6.45166667	2014	26
				2022	30
Málaga	MA	36.73027778	-4.09944444	2023	28
Gandia	GA	39.03361111	-0.18305556	2014	21
				2022	26
Delta del Ebro	DE	40.65027778	0.77888889	2014	31
				2022	29

Table 2. Analysis of Molecular Variance (AMOVA)

Source of variation	%var	F-stat	F-value	Std.Dev.	c.i.2.5%	c.i.97.5%	P-value
Among individual within population	0.517	F_{IS}	0.548	0.002	0.544	0.552	0.001
Among populations within groups	0.003	F_{SC}	0.003	0.000	0.003	0.004	0.001
Among groups	0.054	F_{CT}	0.054	0.001	0.052	0.056	0.001

Table 3. Estimate of temporal effective population size (Estimated Ne) for the two genetic clusters with lower and upper confidence intervals (CI low and CI high).

Per location	**Region**	**Pollak**	**Nei/Tajima**	**Jorde/Ryman**
Gandía	Mediterranean	100.1 (91.9 - 109.5)	107.3 (98.2 - 117.8)	88.9 (85.8 - 92.0)
Ría de Arousa	Atlantic	93.3 (88.1 - 98.8)	103.2 (97.6 - 109.3)	92.0 (86.5 - 98.2)
Delta del Ebro	Mediterranean	70.6 (66.6 - 74.8)	78.0 (73.6 - 82.6)	68.9 (64.6 - 73.8)
Vilarrube	Atlantic	73.5 (69.0 - 78.4)	81.6 (76.6 - 87.1)	73.5 (68.6 - 79.2)

Supplementary Material

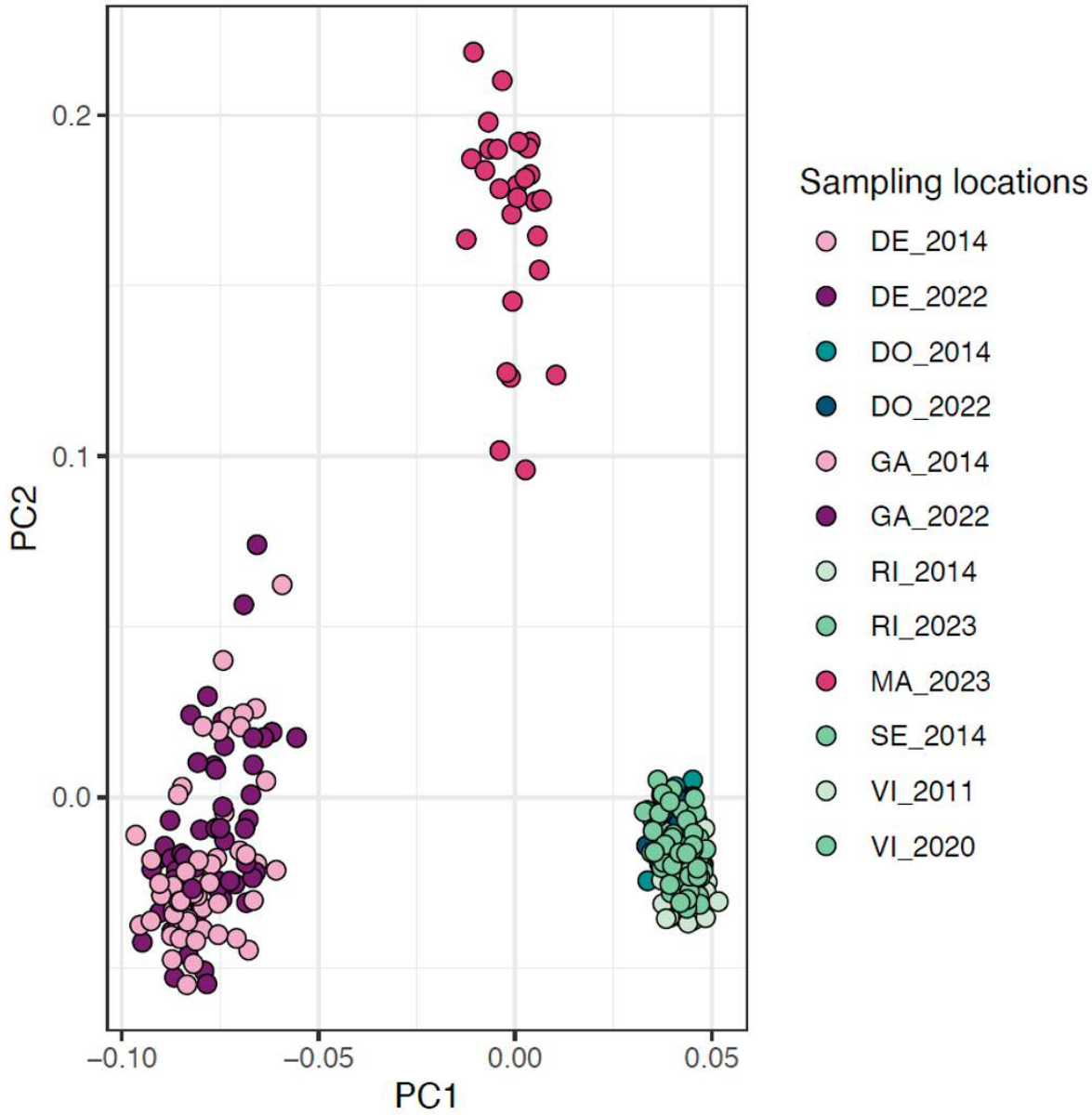

Figure S1. PCAdapt visualization of the outlier detection, linked to the clusters visualized on the principal component analysis.

Figure S2. K-means output showing the two and four genetic clusters using putatively neutral and outlier markers, respectively.

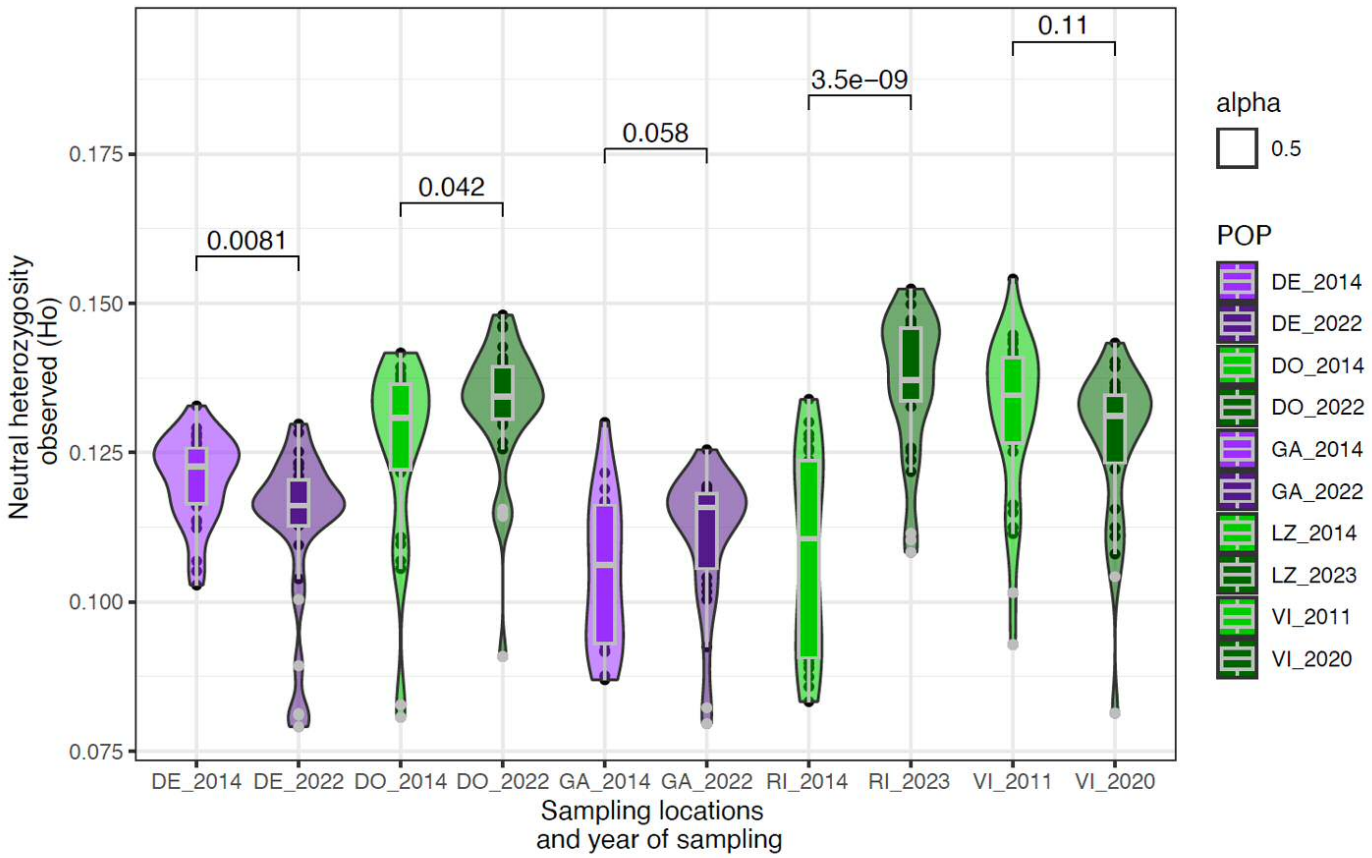

Figure S3. **Genetic diversity changes over time** - Neutral genetic diversity across the year is shown for the five sampling locations with temporal replicates. Statistical tests were performed between temporal replicates, and corresponding P-values are indicated. Each point represents an individual, with colours corresponding to those used in the sampling map.

Table S1. Filtering steps undertaken with subsequent individuals and SNPs remaining per filtering stage.

	SNPs	**Individuals**
Neutral SNPs		
Raw dataset	125,301	378
Remove monomorphic	125,289	378
MAF > 0.05	59,100	378
Loci with less than 35% of missing	8,479	378
Individuals with less than 35% of missing	8,479	331
Hardy-Weinberg Equilibrium in at 70% of the sampling locations (P-value < 0.05)		
LD < 0.2	8,479	331
Removal of related individuals	8,479	325
Removal of outliers	**7,774**	325
Outlier SNPs		
PCAdapt	679	
OUTFLANK	306	
Total unique SNPs	**705**	

Table S2. P-values of the index of genetic differentiation

P-value	**DE_2022**	**DO_2014**	**DO_2022**	**DE_2014**	**GA_2022**	**GA_2014**	**RI_2014**	**VI_2011**	**SE_2014**	**MA_2023**	**VI_2020**	**RI_2023**
DE_2022	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
DO_2014	0.001	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
DO_2022	0.001	**0.05**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
DE_2014	**0.02**	0.001	0.001	NA	NA	NA	NA	NA	NA	NA	NA	NA
GA_2022	0.001	0.001	0.001	0.001	NA	NA	NA	NA	NA	NA	NA	NA
GA_2014	0.001	0.001	0.001	0.001	**0.71**	NA	NA	NA	NA	NA	NA	NA
RI_2014	0.001	0.001	0.001	0.001	0.001	0.001	NA	NA	NA	NA	NA	NA
VI_2011	0.001	0.001	0.001	0.001	0.001	0.001	**0.14**	NA	NA	NA	NA	NA
SE_2014	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	NA	NA	NA	NA
MA_2023	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	NA	NA	NA
VI_2020	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**0.06**	0.001	0.001	NA	NA
RI_2023	0.001	0.001	0.001	0.001	0.001	0.001	0.01	0.001	0.001	0.001	0.001	NA