Interreg Atlantic Area
European Regional Development Fund
EUROPEAN UNION

Acronym:	COCKLES
Title:	Co-Operation for Restoring CocKle SheLlfisheries and its Ecosystem Services in the Atlantic Area
Contract:	EAPA_458/2016

Deliverable 4.4

Cockle population genetics

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Due date of Output	
Actual submission date	

Dissemination level			
☒	**PU** Public	☐	**PP** Restricted to other programme participants
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Acknowledgement

The work described in this report has been funded by the European Commission under the Horizon 2020 Framework Programme.

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1. Executive Summary

Knowledge on how environmental factors shape the genome of marine species is crucial for sustainable management of fisheries and wild populations. The edible cockle (*Cerastoderma edule*) is a marine bivalve distributed along the Northeast Atlantic coast of Europe and is an important resource from both commercial and ecological perspectives. The main objectives for *C. edule* population genetics within COCKLES projects were: (i) assess genetic diversity in Atlantic Area (AA) beds, (ii) analyse temporal and geographic genetic structure, (iii) identify signals of natural selection regarding environmental and biological factors and (iv) define operational conservation units (OCUs) for the species.

To accomplish these objectives, the ACUIGEN research group of the Universidade de Santiago de Compostela (USC) performed a population genomics screening using 2b-RAD genotyping on 9,309 SNPs in 746 specimens pertaining to 22 beds in the Northeast Atlantic provided by COCKLES' partners and collaborators (from South of Portugal to Germany, including British Isles) to ascertain genetic diversity, temporal and geographic population structure at macro- and microgeographical scale (i.e. Galicia region), and footprints of selection regarding environmental and biological factors affecting cockle distribution. Moreover, larval dispersal modelling considering species behaviour and interannual variability in ocean conditions was carried out by UoB (WP5), and this information was used as an essential background to compare genetic information with. At macrogeographical scale (536 individuals, 14 beds) cockle populations in the Northeast Atlantic were shown to be panmictic and displayed low but significant geographical differentiation (F_{ST} = 0.0240; $P < 0.001$) across populations albeit not across generations (F_{ST} = 0), suggesting temporal genetic stability. We identified 441 outlier SNPs (markers under natural selection) related to divergent selection mainly associated with sea surface temperature as the main environmental driver following a latitudinal axis. Two main genetic groups were identified, northwards and southwards of French Brittany, in accordance with larval modelling dispersal identified within WP5 of COCKLES project, which demonstrated a barrier for larval dispersal linked to the Ushant front. Further genetic subdivision was observed using outlier loci and considering larval behaviour. The northern group was divided into the Irish/Celtic Seas and the English Channel/North Sea,

while the southern group was divided into three subgroups (i.e. Biscay Gulf, North-western Spain (Galicia) together with Northern Portugal, and South of Portugal). Refined microgeographic analyses along Galician coast (332 individuals, 10 beds) identified the presence of a single panmictic population unit (F_{ST} = 0.0019; P > 0.050), suggesting no influence of Cape Finisterre as biogeographical barrier. Currently, new samples from the British Isles and France, where barriers have been described (i.e. Celtic Sea and English Channel region), are being analysed for a more comprehensive picture. Therefore, at least two main OCUs northwards and southwards French Brittany can be defined as basic management strategy, although more ambitious one would include at least five units (i.e. (i) Irish/Celtic Sea; (ii) English Channel/North Sea; (iii) Bay of Biscay; (iv) northwest Spain and north Portugal; and (v) south Portugal). This information represents the baseline for management of cockles, designing conservation strategies, founding broodstock for depleted beds, and producing suitable seed for aquaculture production.

2. Introduction

The genetic structure of marine species and patterns of connectivity between discrete populations are central to their health and resilience to external pressures such as parasites and pathogens (Rowley et al., 2014), pollution, sustainable management and exploitation, and climate change over ecological and evolutionary timescales (Cowen & Sponaugle, 2009; Burgess et al., 2014). Detection of genetic structure in marine species remains challenging due to the often large effective population sizes and high levels of gene flow facilitated by the scarcity of physical barriers, which lead to genomic homogenization (Danancher & Garcia-Vazquez, 2011; do Prado et al., 2018; Zhao et al., 2018). However, genetic differentiation can be enhanced by oceanic features such as current systems, fronts, gyres and eddies (Nielsen et al., 2004; Blanco-González, et al., 2016; Vera et al., 2016; Xuereb et al., 2018), and natural selection in response to environmental variation (Vilas et al., 2015; Clucas et al., 2019; Jiménez-Mena et al., 2020). Distinguishing between neutral and adaptive genetic variation in the marine landscape has become a central issue in conservation biology, allowing for interpreting genetic variation in both historical/demographic and adaptive terms (Nielsen et al., 2009; Bernatchez, 2016). This information is essential for identifying broodstock with the necessary genetic diversity for conservation and breeding programs in marine aquaculture (do Prado et al., 2018; Hughes et al., 2019).

The edible cockle, *Cerastoderma edule*, is a bivalve mollusc naturally distributed along the Northeast Atlantic coast, from Senegal to Norway, inhabiting intertidal soft sediments (Hayward & Ryland, 1995). The species plays a crucial role as a food source for birds, crustaceans, and fish (Norris et al., 1998). Moreover, the species is highly appreciated for cuisine, and its main fisheries are located in Ireland, the United Kingdom, France, Spain and Portugal, where its commercialisation represents employment of thousands of collectors, processors and sellers (http://www.cockles-project.eu/). *Cerastoderma edule* is dioecious and can live up to ten years with a fast sexual maturation (reached in its first year) and high fecundity (Honkoop & van der Meer, 1998), with reproductive periods spanning from late spring to mid-autumn (Malham et al., 2012; Mahony et al., 2020). Larvae are planktonic and remain in the water column for around 30 days, which allows for passive larval dispersal by

ocean currents that drive connectivity and gene flow between populations spread along the Northeast Atlantic coast (de Montaudouin et al., 2003; Dare et al., 2004).

The genetic structure of *C. edule* across the Northeast Atlantic has been studied over the last forty years. Pioneering studies using allozymes identified genetic differences between populations located on either side of the English Channel (collected in Wales, France and The Netherlands; see Beaumont et al., 1980), but also a high connectivity and gene flow from France to Denmark (Hummel et al., 1994). Mitochondrial DNA (mtDNA) sequencing carried out on wider sampling (from Morocco to Russia) revealed the presence of two major mtDNA groups: a northern and southern group, suggesting the presence of a northern cryptic refugia for the species (Krakau et al., 2012; Martínez et al., 2015). Studies using microsatellite loci showed high homogeneity in the southern beds from Portugal and Spain (Martínez et al., 2013), while two main clusters were identified in the northern area in the British Isles and the North Sea. In summary, three major areas were defined from microsatellite data: (i) a southern region (Morocco, Portugal, Spain and French beds to the English Channel); (ii) Ireland, Great Britain and southern North Sea (The Netherlands and Germany); and (iii) a northern group (Scotland, Denmark, Norway and Russia) (Martínez et al., 2015). However, the low amount of microsatellite markers used have greatly limited the investigation on local adaptation and population connectivity at the fine scale necessary for the appropriate management of the exploited species (Bernatchez et al., 2017).

Recently, Coscia et al. (2020) analysed the genetic structure and connectivity among cockle populations within the Celtic/Irish seas using data from RADseq and a population genomics approach in combination with information on ocean conditions and larval dispersal modelling. They identified a significant genetic differentiation in a small geographical area (three groups; F_{ST} = 0.021) mainly driven by salinity, larval dispersal by oceanographic currents and geographical distance. These results show that a finer structure underlies cockle distribution in the Northeast Atlantic and that a genomic scan covering southern and northern beds taking as background the dispersal of larvae in the area is necessary to understand how the species is structured for its appropriate management.

Within COCKLES deliverable 4.4 “cockle population genetics”, we used a 2b-RADseq genotyping by sequencing (GBS) approach to assess the genetic structure of *C. edule* along the Northeaster Atlantic coast considering environmental drivers and larval dispersion models data (in collaboration with University of Bangor within COCKLES WP5) with the aim of providing essential information for the sustainable management of its natural resources. Major regions previously identified with microsatellites were confirmed (including no differentiation along Galician coast), but refined information was obtained, mostly in agreement with the ocean dynamics and resulting larval dispersal patterns across the Northeast Atlantic.

3. Materials and methods

3.1. Sampling

A total of 746 cockles from 22 natural beds distributed along the Northeast Atlantic Coast (Fig. 1) were collected during the period 2017-2019 and stored in 100% ethanol (Table 1). Fourteen beds (546 individuals) were used for macrogeographical analysis while the ten restricted to Galicia (332 individuals) were used for microgeographical analysis (Table 1, Fig. 1). Temporal replicates to analyse genetic stability across generations were obtained for six beds in 2017 and 2018 (Table 1). Specimens collected belonged to the 0+ year age class to avoid generation overlapping between consecutive year cohorts.

Table 1. Cockle beds used in the present study. Bed name, cohort year, Geographical coordinates (Lat: Latitude; Long: Longitude), bed code (CODE), sampling size (N, between parentheses number of individuals finally used after quality filtering), and study where bed was included (macro: macrogeographical analysis; micro: microgeographical analysis)

Bed name	Year	Lat (deg N)	Lon (deg E)	Code	Country	N inicial	N	Study
Sylt	2017	54.814	8.298	ASCE_17	Germany	22 (22)	22	macro
Texel	2018	53.004	4.771	NTX_18	Netherlands	21 (21)	21	macro
Dee Estuary	2017	53.343	-3.174	WDE_17	Wales	30 (28)	28	macro
Dee Estuary	2018	53.343	-3.174	WDE_18	Wales	30 (30)	30	macro
Burry	2017	51.643	-4.166	WBY_17	Wales	30 (30)	30	macro
Burry	2018	51.643	-4.166	WBY_18	Wales	30 (30)	30	macro
Dundalk Bay-Annagassan	2018	53.884	-6.341	IDA_18	Ireland	29 (29)	29	macro
Dundalk Bay-Cooley	2018	53.996	-6.287	IDC_18	Ireland	22 (22)	22	macro
Somme Bay	2017	50.201	1.627	FBS_17	France	30 (30)	30	macro
Somme Bay	2018	50.201	1.627	FBS_18	France	31 (31)	31	macro
ARCACHON BAY	2017	44.580	-1.238	FAR_17	France	30 (30)	30	macro
BARQUEIRO, O	2017	43.722	-7.701	SBA_17	Spain	30 (30)	30	micro
MIÑO	2017	43.361	-8.206	SMI_17	Spain	30 (30)	30	micro
ANLLÓNS	2017	43.220	-8.943	SAN_17	Spain	30 (29)	29	micro
Ría de NOIA	2017	42.790	-8.923	SNO_17	Spain	30 (30)	30	macro, micro
Ría de NOIA	2018	42.790	-8.923	SNO_18	Spain	30(30)	30	macro, micro

LOMBOS DO ULLA	2017	42.629	-8.775	SLO_17	Spain	30 (30)	30	macro, micro
LOMBOS DO ULLA	2018	42.629	-8.775	SLO_18	Spain	32 (32)	32	macro, micro
SARRIDO	2017	42.507	-8.826	SSA_17	Spain	30 (30)	30	micro
VILANOVA	2017	42.561	-8.831	SVI_17	Spain	30 (25)	25	micro
CAMPELO	2017	42.421	-8.685	SCA_17	Spain	30 (30)	30	micro
MOAÑA	2017	42.286	-8.730	SMO_17	Spain	30 (19)	19	micro
Baiona	2018	42.117	-8.822	SBI_18	Spain	30 (17)	17	micro
Ria de Aveiro	2017	40.623	-8.739	PRA_17	Portugal	30 (30)	30	macro
Tejo Estuary	2018	38.767	-9.033	PTE_18	Portugal	8 (8)	8	macro
Sado Estuary	2019	38.450	-8.717	PSA_19	Portugal	20 (20)	20	macro
Ria Formosa	2017	36.998	-7.830	PRF_17	Portugal	30 (30)	30	macro
Ria Formosa	2018	36.998	-7.830	PRF_18	Portugal	30 (23)	23	macro

The study area covers the Northeast Atlantic from southeast Portugal to northeast Ireland and the southern North Sea (Fig. 1). This area is divided into several oceanographic regions: Iberian coastal waters, the Bay of Biscay, the English Channel, the Celtic Sea, the Irish Sea and the North Sea. These regions are to some extent discrete units with limited oceanographic connectivity between them resulting from either divergent coastal currents or frontal currents generated during summer heating (Galparoso et al., 2014). During summer months, when upwelling is a prominent feature along the Galician coast (NW Spain), the Portuguese coastal currents transports surface waters southward along the Galician and Portuguese west coast, whereas during the winter months the Iberian Poleward Current shoals and moves surface waters northwards (Teles-Machado et al., 2016). Along the northern coast of Spain, a strong westward transport develops during the summer months which changes direction during the winter months and links into the slope current along the Amorican and Aquitaine Shelf (W France). The coastal circulation along the French coast of the Bay of Biscay is characterised by northward transport by the Iberian Poleward Current during the winter which reverses in direction and reduces in strength during the summer months (Charria et al., 2013). Tidal mixing fronts separating mixed from seasonally stratified

waters form in early summer and extend into the autumn at the entrance to the English Channel (Ushant Front) (Sournia et al., 1998) and between southwest Wales and southeast Ireland (Celtic Sea Front). From late spring to early autumn a current system develops in the Celtic Sea that transports waters from southwestern Britain via the frontal jet associated with the Celtic Sea Front to the south and west coasts of Ireland as the Irish coastal current (Horsburgh et al., 1998; Brown et al., 2003; Fernand et al., 2006).

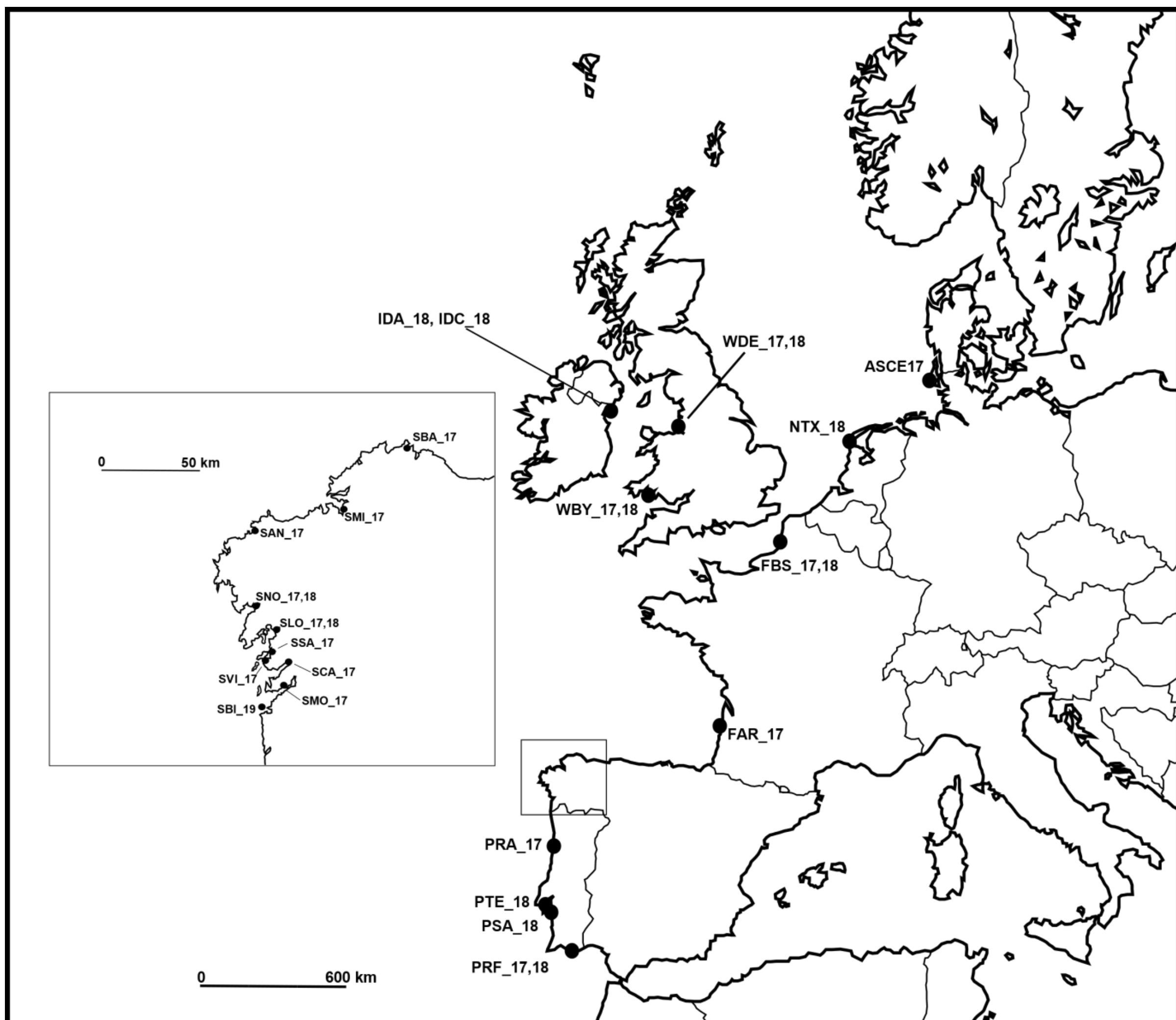


Figure 1. Distribution of cockle beds along Atlantic Area. Bed codes are shown on Table 1.

3.2. Laboratory procedures, identification and genotyping of Single Nucleotide Polymorphisms (SNPs)

Total DNA was extracted from gill tissue samples using the e.Z.N.A. E-96 mollusc DNA kit (OMEGA Bio-tek), following manufacturer recommendations. SNP identification and selection, as well as genotyping and validation protocols followed those described by Maroso et al. (2019), an study carried out to develop a molecular tool to identify *C. edule*, *C. glaucum* and their potential hybrids. This was an additional action within COCKLES WP4.4 taking advantage of the genomic resources obtained. Briefly, AlfI IIb restriction enzyme (RE) was used to construct the 2b-RAD libraries, which were evenly pooled for sequencing in Illumina Next-seq 500 including 90 individuals per run. The recently assembled cockle's genome (Tubío et al., unpublished data) was used to align reads from each individual using Bowtie 1.1.2 (Langmead et al., 2009), allowing a maximum of three mismatches and a unique valid alignment (-v 3 -m 1). Stacks 2.0 (Catchen et al. 2013) was then used to call SNPs and genotype a common set of markers in the sample set, applying the marukilow model with default parameters in the gstacks module of Stacks 2.0. This SNP panel was further filtered following additional criteria: i) genotyped in > 60% individuals; ii) minimum allele count (MAC) ≥ 3; iii) conformance to Hardy-Weinberg equilibrium (HWE), i.e. loci with significant ($P < 0.05$) F_{IS} values (Weir & Cockerham, 1984) detected in at least 25% of populations were removed; and iv) selection of the most polymorphic SNP in each RAD-tag.

3.3. Genetic diversity and population structure

Genetic diversity (i.e. mean number of alleles per locus (Na), observed (Ho) and expected (He) heterozygosities), departure from Hardy–Weinberg equilibrium (HWE) and intrapopulation fixation index (F_{IS}) were estimated for each bed using GENEPOP v4.0 (Rousset, 2008) and ARLEQUIN v3.5 (Excoffier & Lischer, 2010). Linkage disequilibrium between pairs of loci regarding physical distance was estimated with r^2 across the cockle genome using the program PLINK 1.9 (Chang et al., 2015; http://www.cog-genomics.org/plink/1.9) and its significance calculated through exact tests over genotypic contingency tables using GENEPOP v4.0.

Global and pairwise relative coefficients of population differentiation (F_{ST}) between cockle beds were calculated with ARLEQUIN v3.5 using 10,000 permutations to test for significance. The number of genetically homogenous population units (K) was estimated using the variational Bayesian clustering method implemented in the package fastSTRUCTURE v2.3.4 (Raj et al., 2014) for K = 1 – 21 and K = 1-13 for macrogeographical and microgeographical analysis, respectively, with an admixture ancestry model, convergence criterion of 1 x 10-7, five cross-validated sets and the simple prior (flat-beta prior). The most likely number of K was estimated using the “chooseK.py” program. This program gives the best K value and K corresponding with weak population structure in the data using the heuristic scores. Summarized outputs were carried out using the software DISTRUCT 1.1 (Rosenberg, 2004). Discriminant analyses of principal components (DAPC) were run in ADEGENET package (Jombart et al., 2010; Jombart & Ahmed, 2011) for the R platform (R Development Core Team, 2014; http://www.r-project.org). Data were transformed using PCA (Principal Component Analysis) and an appropriate number of principal components (PC) and discriminant functions (DF) were retained to explain > 90% of variance. Analyses of molecular variance (AMOVA) to study the distribution of genetic variation within (F_{SC}) and among (F_{CT}) bed groups obtained from fastSTRUCTURE were carried out with the program ARLEQUIN v.3.5 and their significance tested with 10,000 permutations. Isolation by distance (IBD) was evaluated through the correlation between geographical (measured as the shortest ocean distance) and genetic (measured as FST/1-FST; Rousset, 1997) distance matrices evaluated with a Mantel test with 10,000 permutations using NTSYS v.2.1 (Rohlf, 1993). All these analyses were performed both with the complete SNP dataset (9,309 markers) and the detected divergent outlier loci dataset (441 markers, see Results section for outlier description).

3.4. Outlier tests and gene mining

The Bayesian F_{ST}-based method implemented in BAYESCAN v2.01 (Foll & Gaggiotti, 2008) was used to identify outlier loci potentially under selection in the whole Northeast

Atlantic with samples grouped by beds. BAYESCAN was run using default parameters (i.e. 20 pilot runs; prior odds value of 10; 100,000 iterations; burn-in of 50,000 iterations and a sample size of 5,000). Loci with a False Discovery Rate (FDR, q-value) < 0.05 were considered outliers. RAD-tags including divergent outlier SNPs were mapped in the *C. edule* assembled genome (Tubío et al., unpublished) and used as landmarks for mining the genome to identify candidate genes related to selective drivers. To establish the genome windows for mining, linkage disequilibrium (LD) was evaluated across the whole genome and between consecutive outlier loci at specific genomic regions. It was expected that those regions under selection showed a higher LD than the average due to selective sweeping. LD was represented against physical distance for all pairs of markers across the whole genome, between markers separated up to 500 kilobases (kb) in each bed and up to 5,000 kb in the whole cockle collection of the Northeast Atlantic, respectively. The corresponding r^2 values were averaged within 1 kb and 50 kb genomic windows within each bed and for the whole collection, respectively. Considering this information, and following a conservative criterion, windows of ± 200 kb were established around outlier markers in the selected genomic regions for mining. For this, the most consistent genomic regions related to divergent selection were selected according to the following criteria: i) the presence of two or more consecutive outliers in a region < 150 kb; and ii) the existence of a significant LD between them or above the maximum average genomic LD. Candidate genes for divergent selection included in these genomic windows were identified and annotated using the cockle transcriptome (Pardo et al., unpublished) and genome (Tubío et al., unpublished). Reactome and KEGG biological and molecular enriched pathways associated with these genes were retrieved from Kobas 2.0 using *Homo sapiens* and *Drosophila melanogaster* as background (Xie et al., 2011).

3.5. Landscape analyses

Genetic differentiation explained by the different spatial and abiotic factors was studied following a canonical redundancy analysis (RDA) using the VEGAN software (Oksanen, 2015) in R, using only beds included on macrogeographical analysis. For each bed (for those with

temporal replicates, only information for the cohort 2017 was used), allele frequencies were estimated with ADEGENET package in R (Jombart & Ahmed, 2011) using the "makefreq" option applied on the ADEGENET "genpop" file. Loci with missing values were excluded from the analysis. Latitude and longitude together with the following abiotic factors were available for all the beds except for ASCE_17 (Sylt-Germany): sea surface temperature (SST, ºC); sea bottom temperature (SBT, ºC); sea surface salinity (SSS, psu); sea bottom salinity (SBS, psu); bottom shear stress (BSS, $N \cdot m^{-2}$); net primary productivity (NPP, $mg \cdot m^{-3} \cdot day^{-1}$) (Table 2). Monthly information for all these abiotic factors was retrieved from the IBI_REANALYSIS_PHYS_005_002 model and the IBI_REANALYSIS_BIO_005_003 model , for the period 2014-2018 (Sotillo et al., 2015, 2020) The nearest model cell with water was taken to extract the data. Then, both annual and spawning season (i.e. from April to August, see Malham et al., 2012) averages were calculated for each bed.

Table 2. Environmental information for the *C. edule* beds analysed (macrogeographical analysis). Data were monthly collected from the IBI_REANALYSIS_PHYS_005_002 and IBI_REANALYSIS_BIO_005_003 models for the last five years (2014-2018). The nearest model cell with water was taken to extract the data. Codes are described on Table 1. SST: Sea Surface Temperature; SBT: Sea Bottom Temperature; SSS: Sea Surface Salinity; SBS: Sea Bottom Salinity; BSS: Bottom Shear Stress; NPP: Net Primary Productivity.

	Year Mean Value						Reproductive season values					
Code	SST (ºC)	SBT (ºC)	SSS (psu)	SBS (psu)	BSS (N/m^2)	NPP (mg.m-3.day-1)	SST (ºC)	SBT (ºC)	SSS (psu)	SBS (psu)	BSS (N/m^2)	NPP (mg.m-3.day-1)
ASCE17	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
NTX_18	12.16	12.11	30.26	30.41	0.00612	124.8	14.66	14.54	30.55	30.75	0.00391	205.2
WDE_17	12.03	12.04	31.00	31.00	0.00063	220.8	15.44	15.52	31.26	31.26	0.00079	386.7
WDE_18	12.03	12.04	31.00	31.00	0.00063	220.8	15.44	15.52	31.26	31.26	0.00079	386.7
WBY_17	12.70	12.71	30.09	30.09	0.00016	117.7	15.28	15.31	30.15	30.16	0.00012	175.9
WBY_18	12.70	12.71	30.09	30.09	0.00016	117.7	15.28	15.31	30.15	30.16	0.00012	175.9
IDA_18D	11.43	10.38	33.27	34.01	0.00583	38.1	12.81	10.23	33.35	34.13	0.00130	51.2
IDC_18D	11.21	10.49	30.69	33.95	0.00385	37.3	12.54	10.66	31.03	34.10	0.00152	47.6
FBS_17	13.02	13.01	34.83	34.83	0.00692	70.3	15.47	15.47	34.91	34.91	0.00511	94.8
FBS_18	13.02	13.01	34.83	34.83	0.00692	70.3	15.47	15.47	34.91	34.91	0.00511	94.8
FAR_17	16.06	13.68	33.55	34.56	0.00422	33.0	18.08	13.81	33.17	34.67	0.00143	37.8
SNO_17	14.55	12.81	35.09	35.30	0.01103	52.9	15.15	12.47	35.19	35.43	0.01028	73.8
SNO_18	14.55	12.81	35.09	35.30	0.01103	52.9	15.15	12.47	35.19	35.43	0.01028	73.8
SLO_17	15.17	14.65	33.69	33.76	0.00017	228.2	16.71	15.66	34.04	34.15	0.00021	318.1
SLO_18	15.17	14.65	33.69	33.76	0.00017	228.2	16.71	15.66	34.04	34.15	0.00021	318.1
PRA_17	15.40	13.59	34.51	35.11	0.01118	106.4	16.28	13.18	34.74	35.27	0.01217	148.3

PTE_18	15.70	14.78	33.48	35.11	0.00175	91.8	16.03	14.48	34.05	35.39	0.00200	126.1
PSA_19	15.52	14.06	35.56	35.71	0.00646	61.1	15.41	13.62	35.62	35.79	0.00690	87.8
PRF_17	16.87	14.42	35.45	35.87	0.04639	42.0	17.48	14.23	35.72	35.90	0.05500	55.3
PRF_18	16.87	14.42	35.45	35.87	0.04639	42.0	17.48	14.23	35.72	35.90	0.05500	55.3

ANOVA was performed to test the significance of the variance associated to the different variables using 1,000 random permutations with VEGAN. Variance inflation factor (VIF) was estimated to explore collinearity (correlation) among landscape variables in the dataset. VIF values higher than 10 denote important collinearity problems, values from 5 to 10 moderate problems, while values lower than 5 indicate no collinearity problems (Marquardt, 1970). Different models were adjusted following a forward selection process with the PACKFOR package in R (Dray et al., 2009). This selection process corrects for highly inflated type I errors and overestimated amounts of explained variation (Vandamme et al., 2014). Thus, the reduced panel of explanatory variables is used to recalculate the total proportion of genetic variation in the variance partitioning. The weight of the different loci on the significant environmental vectors was obtained using VEGAN. All these analyses were performed separately for the complete and the divergent outlier SNP datasets taking into account only beds included within macrogeographical analysis.

4. Results

4.1. SNP genotyping and genetic diversity within beds

A total of ~3,000 M raw reads were initially analysed (~3.7 M reads per sample). After quality and population filtering and alignment, the number of retained molecular markers (i.e. SNPs) for the analysis was 9,309.

Genetic diversity was estimated as the average observed (Ho) and expected heterozygosity per locus (He). Ho ranged from 0.070 in Sylt – ASCE_17 and Burry – WBY_18 to 0.079 in Ría de Noia – SNO_17, Ría Formosa – PRF_17 and PRF_18 (mean ± SD = 0.074 ± 0.003), while He from 0.077 in Sylt – ASCE_17 and Burry –WBY_18 to 0.088 in SNO_17 (mean= 0.082 ± 0.003) (Table 3). F_{IS}, which estimates the deviation from Hardy-Weinberg proportions within populations, was positive but not significant, so random mating sustains in all cockle populations. As expected, when a polymorphic criterion was considered (Minimum Allele Frequency (MAF) > 0.010), genetic diversity increased and its range enlarged: Ho from 0.134 in Lombos do Ulla – SLO_18 to 0.271 in Ría Formosa – PTE_18 (mean ± SD = 0.157 ± 0.027) and He from 0.153 in Lombos do Ulla – SLO_18 to 0.290 in Ría Formosa – PTE_18 (mean ± SD = 0.175 ± 0.027). This range widening was mainly due to the Tejo Estuary, Portugal, which represented an outlier population with both estimators much higher than in the other populations. Similar to the F_{IS} estimations considering all SNPs, their values were positive and non-significant in all cases (< 0.127; Table 1). Interestingly, the two Lombos do Ulla – SLO samples in Galicia severely affected by a *Marteilia cochilia* parasite outbreak showed the lowest genetic diversity (see Discussion).

Table 3. Diversity levels of cockle beds analysed. Code, sampling size (N), observed homozygosity (Ho), expected heterozygosity (He) and inbreeding coefficient (F_{IS}) for all dataset and polymorphic loci are shown.

		All dataset			Polymorphic loci (MAF > 0.010)		
Code	**N**	**Ho**	**He**	**F_{IS}**	**Ho**	**He**	**F_{IS}**
ASCE_17	22	0.070	0.077	0.089	0.172	0.191	0.099
NTX_18	21	0.075	0.078	0.034	0.183	0.193	0.052

WDE_17	28	0.071	0.078	0.079	0.165	0.182	0.093
WDE_18	30	0.075	0.083	0.099	0.153	0.172	0.110
WBY_17	30	0.073	0.081	0.092	0.148	0.165	0.103
WBY_18	30	0.070	0.077	0.098	0.157	0.176	0.108
IDA_18	29	0.074	0.081	0.083	0.159	0.176	0.097
IDC_18	22	0.077	0.084	0.084	0.173	0.190	0.089
FBS_17	30	0.072	0.080	0.110	0.146	0.166	0.120
FBS_18	31	0.072	0.079	0.084	0.149	0.165	0.097
FAR_17	30	0.074	0.083	0.111	0.138	0.157	0.121
SBA_17	30	0.076	0.085	0.104	0.139	0.157	0.115
SMI_17	30	0.074	0.082	0.104	0.138	0.155	0.110
SAN_17	29	0.073	0.082	0.101	0.142	0.160	0.113
SNO_17	30	0.079	0.088	0.098	0.139	0.155	0.103
SNO_18	30	0.072	0.081	0.118	0.137	0.158	0.133
SLO_17	30	0.076	0.086	0.114	0.136	0.155	0.123
SLO_18	32	0.073	0.083	0.119	0.134	0.153	0.124
SSA_17	30	0.075	0.083	0.101	0.139	0.157	0.115
SVI_17	25	0.072	0.081	0.113	0.152	0.173	0.121
SCA_17	30	0.073	0.083	0.117	0.138	0.158	0.127
SMO_17	19	0.071	0.078	0.085	0.180	0.198	0.091
SBI_19	17	0.075	0.083	0.091	0.173	0.192	0.099
PRA_17	30	0.072	0.081	0.113	0.141	0.161	0.124
PTE_18	8	0.073	0.078	0.063	0.271	0.290	0.066
PSA_19	20	0.071	0.080	0.108	0.177	0.202	0.124
PRF_17	30	0.079	0.084	0.066	0.146	0.158	0.076
PRF_18	23	0.079	0.086	0.079	0.159	0.174	0.086

4.2. Outlier detection and gene mining

Detection of SNPs under selection using only beds included in macrogeographical analysis was addressed with a BAYESCAN program, which tests whether the genetic differentiation between populations is above or below the neutral background (outlier loci), thus being suggestive of divergent or balancing selection, respectively. BAYESCAN analysis detected a total of 460 outlier loci potentially under selection, 19 under balancing selection and 441 under divergent selection in the whole Northeast Atlantic (Table 4). The set of 441 loci under divergent selection was used along with the whole SNP dataset (9309 SNPs) for assessing patterns of structure under environmental drivers versus neutral factors (population size, larval dispersion) to identify candidate genes and functions associated with adaptation.

All the outliers were mapped against the *C. edule* genome (Tubío et al., unpublished), being mostly distributed across the 19 mega-scaffolds of its assembly corresponding to the number of haploid chromosomes of the cockle's karyotype (Insua & Thiriot-Quiévreux, 1992; Table 4). This information was used to estimate LD between adjacent markers regarding physical distance across the cockle's genome within each population as well as in the whole cockle collection of the Northeast Atlantic (Fig. 2). Results showed that LD was low both within each population as well as in the whole studied area, and the highest average LD (r^2; the square of the correlation coefficient to check for the non-random association of alleles at two loci) was always below 0.050, even for very short distances. Within each bed, LD was not significant for nearly all pairwise comparisons, in part due to the limited sample size (N ~ 30), but even when all data from the Northeast Atlantic was pooled (580 individuals), LD was mostly negligible and not significant above 50 kb on average. Using the whole population data, the most consistent genomic regions under divergent selection were defined by consecutive outlier markers at < 150 kb showing significant LD ($P < 0.05$) or above the maximum average in the studied area ($r^2 = 0.03$; Fig. 2) and were located at chromosome 1 (C1, four regions), C3 (two regions), and C2, C5, C10, C11 and C14 (one region) (Table 4). These regions comprehended less than 25 kb and LD was significant in nearly all cases ($P < 0.05$), r^2 averaging 0.166 and reaching up to 0.857 in one region of C1. These observations suggest selective sweeps at those regions, to say, selection of favourable haplotypes driven by particular environmental factors determining LD and/or loss of genetic diversity. Despite the poor annotation of the cockle's genome outlined above, the windows evaluated were particularly enriched in Gene Ontology (GO) terms related to neural function and development, immune response and defence, and metabolism and growth. Using the human genome as background, we could identify highly significant enriched molecular and metabolic pathways using Reactome and KEGG databases such as Immune System, Cellular Senescence and Necroptosis (corrected P-value < 0.001); Innate Immune System, Interleukin Signalling, Cell Cycle, Metabolism and Signal Transduction ($P < 0.01$), among others terms; and Insulin Secretion, Cytokine Signalling and Immune System and Lipolysis ($P < 0.05$), among others. Taking *Drosophila melanogaster* transcriptome as background, results were very poor due to the low number of significant

homologies detected (not shown). Although information is still preliminary, results suggest essential biological functions related to adaptation in edible cockle that would deserve further work and that could be essential to understand the environmental factors driving selection in the Northeast Atlantic region.

Table 4. Distribution of outliers along the *C. edule* genome assembly.

	Bayescan Outliers (FDR < 0.05)	
Genomic Location	Balancing selection	Divergent Selection
Mega-scaffold 1	2	40
Mega-scaffold 2	1	33
Mega-scaffold 3		39
Mega-scaffold 4	1	31
Mega-scaffold 5	2	28
Mega-scaffold 6		24
Mega-scaffold 7	2	13
Mega-scaffold 8	3	24
Mega-scaffold 9	2	18
Mega-scaffold 10	1	23
Mega-scaffold 11	1	15
Mega-scaffold 12		22
Mega-scaffold 13	1	25
Mega-scaffold 14	2	14
Mega-scaffold 15	1	16
Mega-scaffold 16		32
Mega-scaffold 17		13
Mega-scaffold 18		4
Mega-scaffold 19		11
Other scaffolds (15)		15
Not found in genome		1
Total	19	441

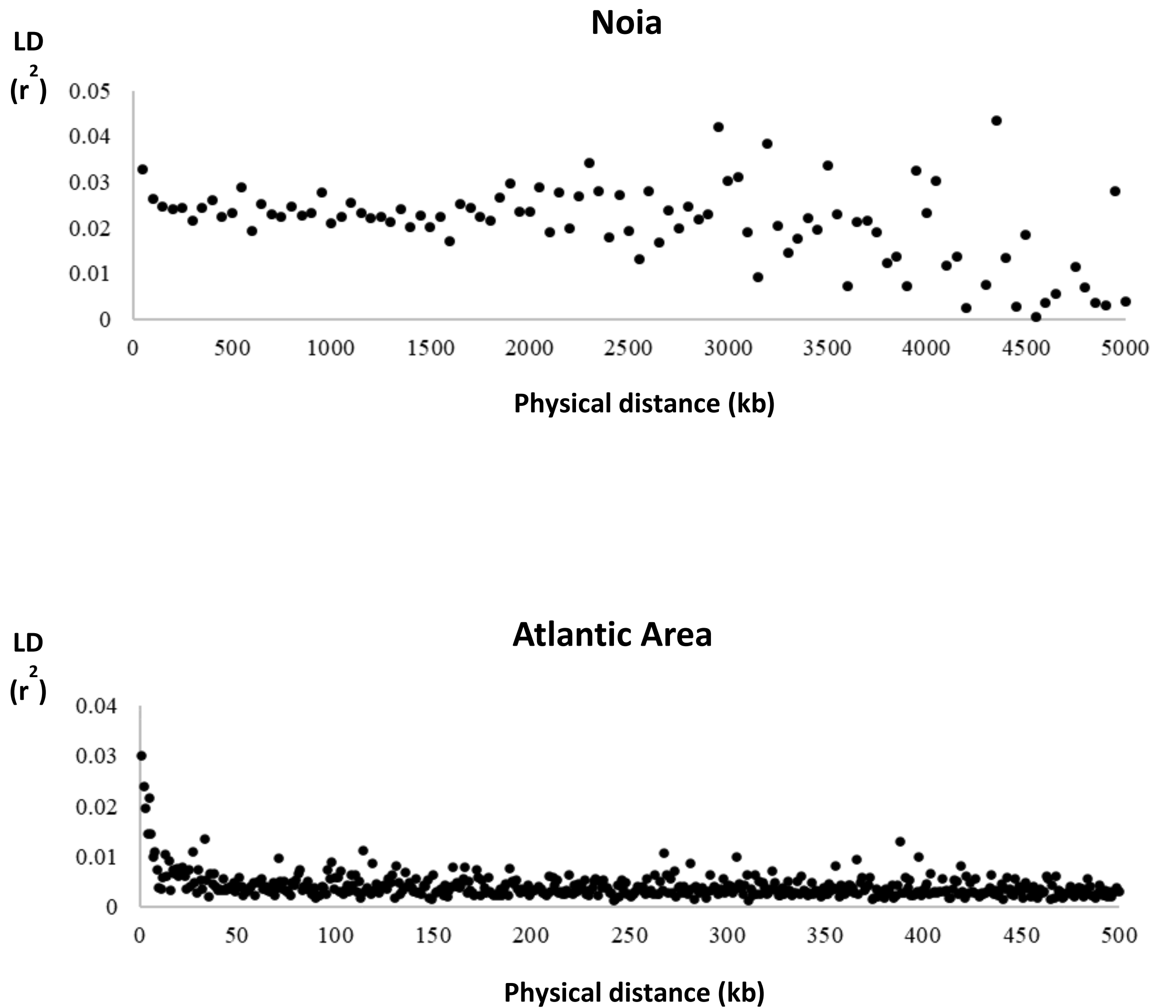


Fig 2. Linkage disequilibrium (r^2) regarding physical distance in the *C. edule* genome in a representative cockle bed (Noia) and using the whole dataset from the Atlantic Area.

4.3. Population structure: temporal and geographical factors

All pairwise F_{ST} values between temporal replicates were non-significant (all F_{ST} values between temporal replicates < 0.0015; all P-values > 0.200), suggesting temporal genetic stability between consecutive cockle's cohorts in the Northeast Atlantic (Table 5). This stability was confirmed when integrating the whole data set using an AMOVA analysis, where the percentage of variation associated to differences among temporal replicates within bed (F_{SC})

was non-significant and negligible, while the percentage among sampling sites (F_{CT}) was highly significant (P < 0.001) and higher than 3% (Model I, Table 6).

Table 5. Pairwise F_{ST} values among analysed *C. edule* beds using complete marker dataset (below diagonal) and divergent outliers (above diagonal) for macrogeographical analysis. All P-values were significant (P < 0.010), except those for the values shown in bold letters. Bed codes are shown on Table 1.

	ASCE_17	NTX_18	WDE_17	WDE_18	WBY_17	WBY_18	IDA_18	IDC_18	FBS_17	FBS_18	FAR_17	SNO_17	SNO_18	SLO_17	SLO_18	PRA_17	PTE_18	PSA_19	PRF_17	PRF_18
ASCE_17	0.0000	**0.0042**	0.0127	0.0108	0.0818	0.0637	0.0597	0.0623	0.0851	0.0743	0.1148	0.1267	0.1359	0.1235	0.1340	0.1425	0.1636	0.1660	0.1541	0.1540
NTX_18	**-0.0009**	0.0000	0.0311	0.0320	0.0894	0.0829	0.0867	0.0802	0.0875	0.0797	0.1321	0.1522	0.1593	0.1452	0.1562	0.1608	0.1736	0.1810	0.1718	0.1734
WDE_17	0.0212	0.0253	0.0000	***0.0044***	0.0848	0.0866	0.0819	0.0650	0.1078	0.1015	0.1496	0.1680	0.1693	0.1626	0.1679	0.1665	0.1732	0.1778	0.1849	0.1858
WDE_18	0.0098	0.0220	***-0.0128***	0.0000	0.0795	0.0792	0.0694	0.0564	0.0980	0.0875	0.1410	0.1543	0.1634	0.1522	0.1579	0.1597	0.1717	0.1803	0.1767	0.1782
WBY_17	0.0118	0.0226	0.0108	0.0162	0.0000	***-0.0027***	0.0475	0.0517	0.0472	0.0416	0.1719	0.1771	0.1844	0.1728	0.1817	0.1898	0.2135	0.2216	0.1927	0.1909
WBY_18	0.0121	0.0177	0.0141	**-0.0067**	***-0.0134***	0.0000	0.0475	0.0300	0.0503	0.0469	0.1722	0.1859	0.1838	0.1831	0.1853	0.1864	0.1838	0.1920	0.1854	0.1831
IDA_18	0.0206	0.0284	0.0107	0.0160	0.0253	0.0102	0.0000	**-0.0133**	0.0832	0.0841	0.1619	0.1666	0.1671	0.1661	0.1690	0.1693	0.1827	0.1856	0.1824	0.1809
IDC_18	0.0093	0.0190	0.0025	0.0174	0.0252	**-0.0015**	**-0.0012**	0.0000	0.0723	0.0656	0.1480	0.1409	0.1579	0.1404	0.1511	0.1644	0.1926	0.1986	0.1681	0.1684
FBS_17	**-0.0033**	0.0062	0.0186	0.0279	0.0241	0.0069	0.0322	0.0291	0.0000	***0.0009***	0.1680	0.1861	0.1877	0.1786	0.1900	0.1863	0.1921	0.2040	0.1942	0.1931
FBS_18	**-0.0014**	0.0068	0.0214	0.0218	0.0196	0.0120	0.0276	0.0223	***-0.0009***	0.0000	0.1653	0.1883	0.1845	0.1823	0.1894	0.1846	0.1852	0.1935	0.1921	0.1898
FAR_17	0.0152	0.0268	0.0304	0.0470	0.0405	0.0135	0.0450	0.0453	0.0366	0.0289	0.0000	0.0407	0.0396	0.0344	0.0331	0.0253	0.0234	0.0323	0.0517	0.0534
SNO_17	0.0092	0.0238	0.0249	0.0473	0.0384	0.0040	0.0427	0.0486	0.0373	0.0281	0.0106	0.0000	***0.0011***	**0.0047**	**0.0034**	**-0.0047**	**0.0101**	0.0284	0.0635	0.0656
SNO_18	0.0264	0.0374	0.0407	0.0487	0.0442	0.0283	0.0484	0.0489	0.0417	0.0401	0.0089	***-0.0049***	0.0000	**0.0028**	**-0.0003**	**0.0028**	0.0193	0.0437	0.0629	0.0647
SLO_17	0.0127	0.0252	0.0253	0.0479	0.0392	0.0048	0.0420	0.0475	0.0375	0.0278	0.0097	**0.0002**	**-0.0023**	0.0000	***0.0023***	**-0.0044**	**0.0045**	0.0146	0.0456	0.0495
SLO_18	0.0202	0.0335	0.0345	0.0515	0.0442	0.0182	0.0489	0.0512	0.0419	0.0339	0.0105	**-0.0003**	**-0.0007**	***0.0012***	0.0000	**-0.0016**	**0.0085**	0.0230	0.0497	0.0574
PRA_17	0.0301	0.0385	0.0456	0.0478	0.0429	0.0330	0.0492	0.0453	0.0402	0.0382	0.0026	**-0.0107**	**0.0001**	**-0.0072**	**-0.0028**	0.0000	0.0245	0.0397	0.0547	0.0591
PTE_18	0.0389	0.0402	0.0530	0.0387	0.0436	0.0451	0.0465	0.0379	0.0381	0.0401	**-0.0043**	**-0.0148**	**-0.0005**	**-0.0159**	**-0.0074**	**0.0043**	0.0000	**0.0088**	0.0125	0.0158
PSA_19	0.0395	0.0429	0.0546	0.0400	0.0407	0.0455	0.0496	0.0397	0.0365	0.0396	**-0.0046**	**-0.0161**	0.0036	**-0.0171**	**-0.0070**	0.0070	**0.0016**	0.0000	0.0097	0.0086
PRF_17	0.0270	0.0358	0.0379	0.0495	0.0459	0.0214	0.0491	0.0490	0.0448	0.0371	0.0150	0.0103	0.0135	0.0088	0.0124	0.0108	**-0.0066**	**-0.0088**	0.0000	***0.0052***
PRF_18	0.0208	0.0329	0.0326	0.0483	0.0425	0.0138	0.0461	0.0500	0.0437	0.0343	0.0146	0.0129	0.0141	0.0102	0.0128	0.0078	**-0.0102**	**-0.0158**	***0.0003***	0.0000

Pairwise F_{ST} values were significant between all the studied beds, except for some comparisons involving nearby sampled beds within each country (Table 5). Pairwise F_{ST} ranged from -0.0171 (Lombos do Ulla – SLO_17 vs Sado Estuary – PSA_19) to 0.0546 (Dee Estuary – WDE_17 vs Sado Estuary – PSA_19), with a global F_{ST} value of 0.0240 (P < 0.001). As expected, F_{ST} increased when only divergent outlier loci were considered (global F_{ST} = 0.1157, P < 0.001), ranging from -0.0133 (Dundalk Bay – IDC_18 vs Dundalk Bay – IDA_18) to 0.2216 (Dee Estuary – WDE_17 vs Sado Estuary – PSA_19). A consistent distribution of genetic diversity according to geographical distance was found, confirmed by a significant isolation by distance (IBD) pattern (complete dataset: r = 0.60541, P < 0.001; outlier dataset: r = 0.69568, P < 0.001).

Table 6. AMOVAs for European *C. beds*.

	F-statistic	Variance component	% Variation
Model I – Temporal (6 groups)			
Among locations (F_{ST})	0.03501***	3.58724	3.50
Among sampling sites (F_{CT})	0.03513***	3.59951	3.51
Among temporal replicates within sampling site (F_{SC})	-0.00012 NS	-0.01227	-0.01

Within locations		98.87860	96.50
Model II – STRUCTURE – all marker dataset (K = 2)			
Among locations (F_{ST})	0.03769***	9.14631	3.77
Among fastSTRUCTURE groups (F_{CT})	0.02977***	7.22427	2.98
Among locations within fastSTRUCTURE groups (F_{SC})	0.00816***	1.92204	0.79
Within locations		233.52461	96.23
Model III – STRUCTURE - outliers (K =2 / K = 10)			
Among locations (F_{ST})	0.17195*** / 0.13055***	6.41246 / 4.63671	17.19/13.06
Among fastSTRUCTURE groups (F_{CT})	0.13455*** / 0.12707***	5.01753 / 4.51316	13.45/12.71
Among locations within fastSTRUCTURE groups (F_{SC})	0.04322*** / 0.00399***	1.39493 / 0.12355	3.74/0.35
Within locations		30.87961 / 30.87961	82.81/86.94

NS: Non Significant (P > 0.05)
** P < 0.01
*** P < 0.001

Bayesian clustering analysis performed with fastSTRUCTURE using the complete dataset (i.e. 9,309 SNPs) rendered a value of K = 2 as the most probable population structure (Fig. 3). One group was formed by the northern beds (above 48º N, including the Bay of Somme (France) and north to Germany, Britain and Ireland), while the southern group was constituted by the beds near Arcachon (France) and south to Spain and Portugal (Fig. 3). AMOVA analysis using these two groups indicated that this structuring (F_{CT} = 2.98% of the total genetic diversity) captured close to the 80% of the total differentiation between populations (F_{ST} = 3.77%) (Model II, Table 6). The best K value with the outlier dataset was also 2 and the grouping was identical to that described with the complete dataset. However, the K value for the weak population structure using the heuristic scores provided by fastSTRUCTURE was 10. Among these, seven main groups were well defined: (i) North Sea and English Channel beds to the Bay of Somme (ASCE_17, NTX_18, FBS); (ii) the Dee bed in North Wales (WDE); (iii) Burry bed in South Wales (WBY); (iv) the Irish beds (IDA_18 and IDC_19); (v) the bed near Arcachon (FAR_17); (vi) the Spanish beds together with the northern Portuguese bed (SNO, SLO, PRA_17); (vii) the southern Portuguese beds (PTE_18, PSA_19, PRF) (Fig. 3). AMOVA analyses with this dataset (K = 10) assigned a higher percentage of genetic variation to differences among groups than with the whole dataset (Model III, Table 6). The percentage of variation associated to differences among beds within groups was the lowest (Table 6), confirming their genetic homogeneity, and accordingly, that the main differences among groups were captured (97.3% of the variation among beds). DAPC plots confirmed the results

found with fastSTRUCTURE regarding the main north-south subdivision, but further clustering was suggested within groups. The analysis with the complete dataset showed an important dispersion within each group (Fig. 4A), while the analysis with the outlier dataset clearly identified four main differentiated groups: (i) Celtic and Irish Seas; (ii) North Sea and English Channel; (iii) the Bay of Biscay and Iberian waters to northern Portugal; and (iv) Iberian waters in southern Portugal, and even a more subtle subdivision up to the seven groups observed with STRUCTURE using outlier loci could be devised (Fig. 4B).

Microgeographical analysis along Galician coast did not detect genetic differentiation among the studied Galician beds (global F_{ST} = 0.0019; $P > 0.05$), and the the most probable K value for the region using fastSTRUCTURE was 1. Thus, all pairwise F_{ST} values also resulted very low and non-significant ($P > 0.05$; Table 7), suggesting the presence of one panmictic unit in Galicia, with no effect of Cape Finisterre as biogeographical barrier for *C. edule*).

Table 7. Pairwise F_{ST} values among Galician beds used for the microgeographical analysis. All P-values were non-significant (i.e. $P > 0.05$). Values for temporal replicates are indicated in blod letters. Bed codes are shown on Table 1.

	SBA_17	SMI_17	SAN_17	SNO_17	SNO_18	SLO_17	SLO_18	SSA_17	SVI_17	SCA_17	SMO_17	SBA_19
SBA_17	0.0000											
SMI_17	0.0015	0.0000										
SAN_17	0.0014	0.0018	0.0000									
SNO_17	0.0004	0.0009	0.0003	0.0000								
SNO_18	0.0007	0.0015	0.0004	**-0.0005**	0.0000							
SLO_17	0.0012	0.0010	0.0005	-0.0004	0.0018	0.0000						
SLO_18	0.0014	0.0008	0.0006	0.0001	0.0002	**0.0013**	0.0000					
SSA_17	0.0016	0.0010	0.0009	0.0002	0.0000	-0.0002	0.0001	0.0000				
SVI_17	0.0004	0.0007	0.0002	-0.0024	-0.0005	-0.0010	-0.0007	-0.0011	0.0000			
SCA_17	0.0011	0.0012	-0.0003	-0.0007	0.0003	0.0015	-0.0005	0.0008	-0.0014	0.0000		
SMO_17	0.0020	0.0018	0.0002	-0.0019	0.0005	0.0018	-0.0002	-0.0006	-0.0005	-0.0015	0.0000	
SBA_19	0.0024	0.0023	0.0016	0.0004	0.0016	0.0011	-0.0009	0.0017	-0.0010	-0.0006	0.0022	0.0000

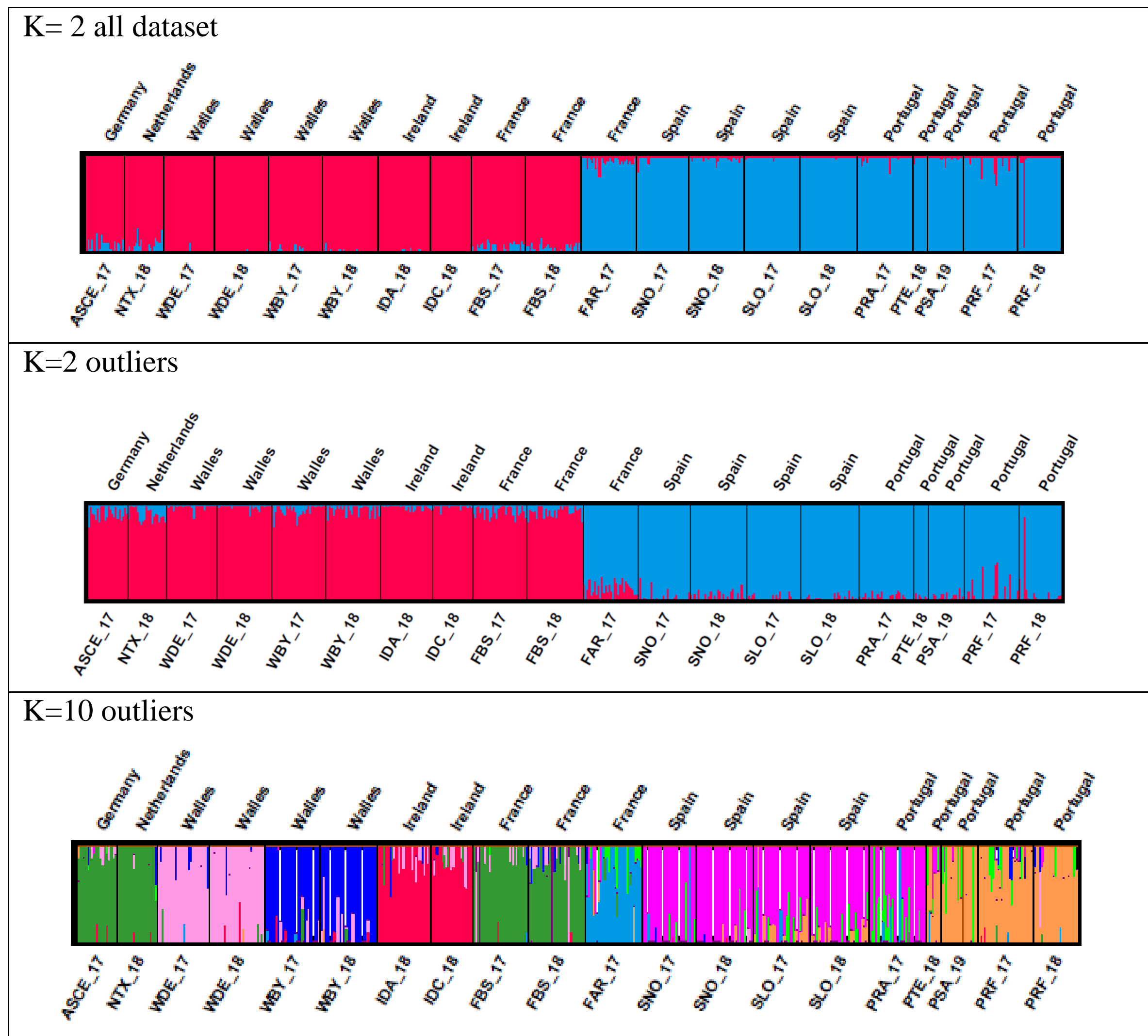
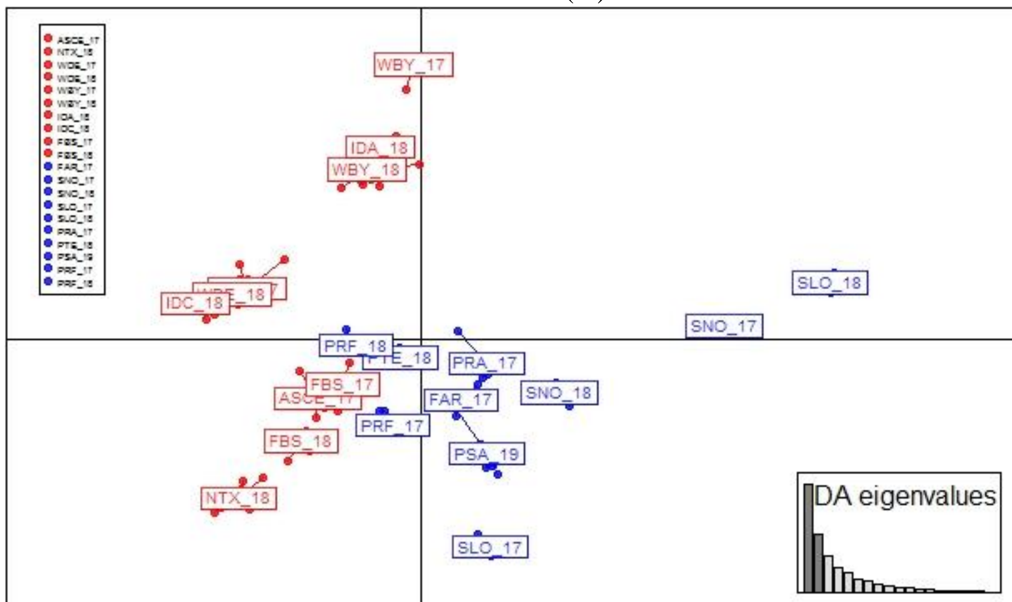


Figure 3. Population structure in the Atlantic Area using fastSTRUCTURE. Each vertical bar represents one individual, and the colour proportion for each bar represents the posterior probability of assignment of each individual to the different clusters (K) inferred by the program. Results obtained with K=2 for all dataset (A), K=2 using divergent outliers (B) and K=10 using divergent outliers (C) are represented. Codes are shown on Table 1.

All dataset (A)



Outliers (B)

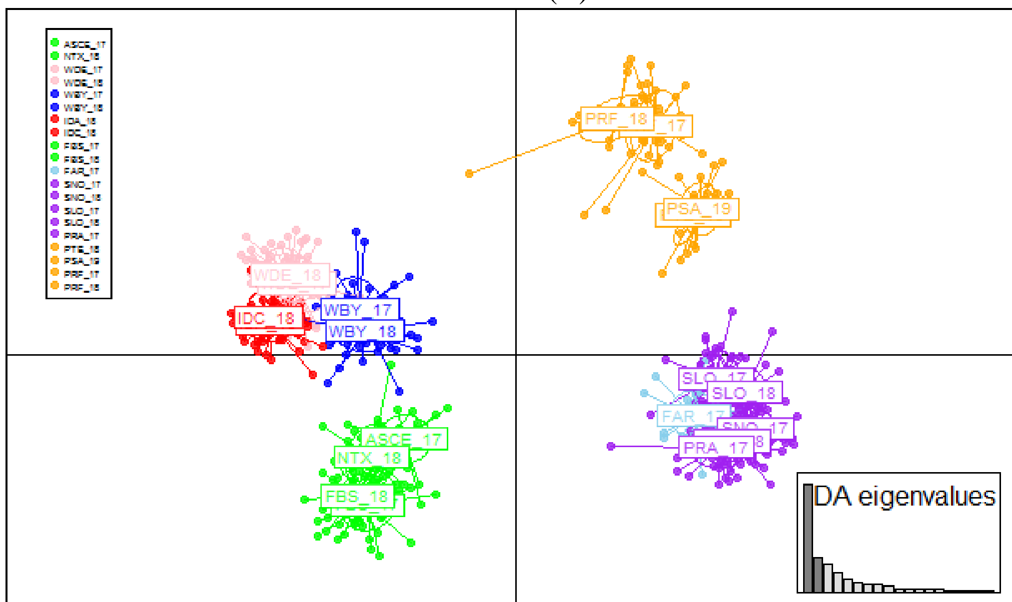


Figure 4. Discriminant Analysis of Principal Components (DAPC) plots. The weight of retained discriminant analysis (DA) eigenvalues to represent > 90% of variance are shown on right bottom box. Results for all dataset (A) and using exclusively divergent outliers (B) are represented. Codes are shown on Table 1.

4.4. Genetic-environmental associations

When all the environmental variables were included in the RDA analyses (measured as their average annual values for abiotic factors), only latitude was suggested as a driver for genetic differentiation, both for complete and outlier datasets (Table 8). Latitude was mainly associated with the first axis (Figs. 5A and 5C), which separated the beds in the two groups previously suggested by fastSTRUCTURE (i.e. northern and southern groups). Despite being non-significant, the remaining variables were also associated with the first axis excluding longitude and net primary productivity (NPP), which were associated with the second axis. When exclusively abiotic variables were used, the sea surface temperature (SST) was suggested as a significant driver (Table 8). SST was also related to the first axis and again separated the cockle beds in the northern and southern groups (Fig. 5B and 5D). However, these results should be taken with caution since most VIF values were > 20 excluding longitude (VIF = 0.55), bottom shear stress (BSS; VIF = 4.70) and net primary production (NPP; VIF = 5.43). When these three variables were evaluated including only one of the variables with collinearity problems each time, all of the latter were significant ($P < 0.050$), except sea bottom temperature (SBT), using the whole and outlier datasets and again related to the differentiation between northern and southern groups (Table 9). Therefore, several environmental variables would be likely shaping the cockle genome in the Northeast Atlantic, including salinity (SSS and SBS), but all of them appeared highly correlated splitting the studied area into northern and southern groups, as observed with the whole SNP dataset. The results obtained using only the spawning season values for the abiotic factors were identical to those found with the average annual values (data not shown).

Table 8. Results of the redundancy analysis (RDA) on *C. edule* beds. Model 1: Forward selection model starting from all landscape variables; Model 2: only from environmental variables. SST: Sea Surface Temperature; SBT: Sea Bottom Temperature; SSS: Sea Surface Salinity; SBS: Sea Bottom Salinity; BSS: Bottom Shear Stress; NPP: Net Primary Productivity.

		9309 SNPs		441 divergent outliers	
Model		**P-value**	**Adjusted R^2**	**P-value**	**Adjusted R^2**
Model 1	Latitude	0.001	0.075	0.002	0.320
	Longitude	0.209		0.215	

	SST	0.295		0.406	
	SBT	0.496		0.489	
	SSS	0.923		0.840	
	SBS	0.909		0.935	
	BSS	0.291		0.328	
	NPP	0.758		0.568	
Model 2	SST	0.002	0.080	0.002	0.314
	SBT	0.299		0.326	
	SSS	0.773		0.662	
	SBS	0.929		0.956	
	BSS	0.286		0.329	
	NPP	0.459		0.343	

Adjusted R^2 and P-value associated to each variable of its selection stage

Table 9. Results of the redundancy analysis (RDA) on *C. edule* beds including only one of the each variable with collinearity problems. SST: Sea Surface Temperature; SBT: Sea Bottom Temperature; SSS: Sea Surface Salinity; SBS: Sea Bottom Salinity; BSS: Bottom Shear Stress; NPP: Net Primary Productivity.

		All SNPs		**Outliers**	
Model		**P-value**	**Adjusted R^2**	**P-value**	**Adjusted R^2**
Latitude Model	**Latitude**	0.001	0.126	0.001	0.432
	Longitude	0.146		0.164	
	BSS	0.129		0.186	
	NPP	0.458		0.420	
SST Model	**SST**	0.001	0.100	0.005	0.324
	Longitude	0.034		0.020	
	BSS	0.224		0.473	
	NPP	0.405		0.406	
SBT Model	**SBT**	0.313	0.054	0.328	0.182
	Longitude	0.024		0.027	
	BSS	0.124		0.406	
	NPP	0.369		0.314	
SSS Model	**SSS**	0.013	0.059	0.018	0.198
	Longitude	0.242		0.206	
	BSS	0.373		0.689	
	NPP	0.574		0.539	
SBS Model	**SBS**	0.033	0.048	0.019	0.171
	Longitude	0.255		0.194	

BSS	0.306	0.561
NPP	0.448	0.386

Adjusted R^2 and p-value associated to any variable of it selection stage

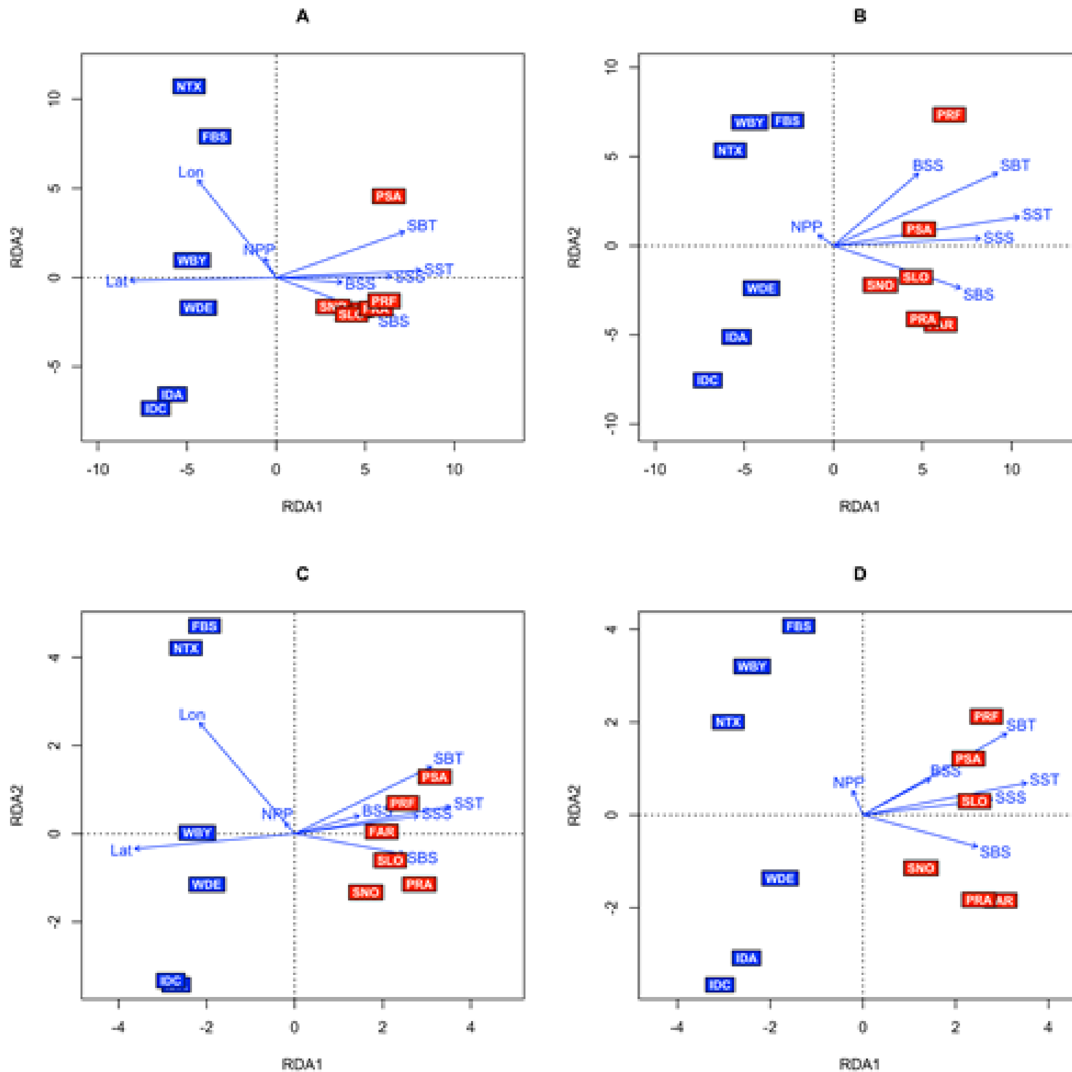


Figure 5. Redundancy analyses (RDA) of *C. edule* samples from the studied area using the complete (A and B) and outliers (C and D) datasets taking into account all landscape variables (Model 1, A and C) and only abiotic factors (Model 2, B and D). SST: Sea Surface Temperature; SBT: Sea Bottom Temperature; SSS: Sea Surface Salinity; SBS: Sea Bottom Salinity; BSS: Bottom Shear Stress; NPP: Net Primary Productivity. Bed codes are shown on Table 1. Colour of the beds corresponds with population units shown on Figure 3 for K = 2.

5. Discussion

5.1. Genetic diversity

Maintenance of genetic diversity is crucial for the adaptation of natural populations to environmental changes. Preservation of connectivity among populations is essential to maintain the genetic diversity within and between populations to counteract the threat of demographic depletion caused by overexploitation or diseases (Frankham et al., 2002). Moreover, genetic diversity existing in the wild is important for commercial species, such as C. *edule*, to support breeding programs for selecting high-value seed for intensive production in particular areas (Lallias et al., 2010; do Prado et al., 2018; Petereit et al., 2018; Vera et al., 2019). Genetic diversity was similar across all cockle beds studied in the AA, especially when no polymorphic filtering criterion was applied (MAF; He range: 0.077-0.088). When a MAF filtering > 0.01 was applied to avoid the bias of low polymorphic loci in accordance with the sample size managed (i.e. 1/(2*highest N) = 0.016; N (SLO_18) = 32 individuals on average), the genetic diversity range widened (0.153 - 0.290), but mostly due to the Tejo Estuary representing an outlier population with much higher He than the rest (He = 0.290). There is not a straightforward explanation for the high genetic diversity in Tejo Estuary, such as admixture of differentiated stocks in that area (Wahlund effect; see below), since the intrapopulation fixation index F_{IS} was not significant and even among the lowest in the samples studied. At the other end of the range, Lombos do Ulla (Galicia) samples showed the lowest genetic diversity in all the studied region, but in this case the serious population depletion caused by the parasite *Marteilia cochillia* that affects this bed since 2012 (Villalba et al., 2014), could be responsible. Moreover, all populations showed a slight heterozygote deficit (F_{IS} > 0) as previously reported in many mollusc species, a fact that has been related to a technical genotyping issue associated with the presence of null alleles (Pino-Querido et al., 2014).

Heterozygosity figures in our study were similar to those reported by Coscia et al. (2020) (He: 0.144 - 0.156), who also applied a MAF filter > 0.01 to characterise genetic diversity in *C. edule* beds from the Celtic and Irish Seas (i.e. southeast Ireland and Wales) using a classical

RADseq technique (Etter et al., 2011). Genetic diversity observed for *C. edule* in COCKLES project was similar to that reported for the sister species *C. glaucum* (He: 0.078 - 0.137; Sromek et al., 2019); however, both cockle species showed lower genetic diversity at genomic scale than other bivalves such as *Placopecten magellanicus* (He = 0.271 ± 0.133, Van Wyngaarden et al., 2017), *Crassostrea virginica* (He ~ 0.300; Bernatchez et al., 2019), *Ostrea edulis* (He ~ 0.300; Vera et al., 2019) and *Crassostrea gigas* (He ~ 0.300; Gutierrez et al., 2017), suggesting the lower genetic diversity to be a particular feature of the genus *Cerastoderma*, although we cannot discard that the different methodologies applied for SNP genotyping in those studies might explain this observation.

Genetic diversity (Ho and He) showed low differences between the two major northern and southern cockle genetic groups identified in the Northeast Atlantic (Mann-Whitney U tests $P > 0.050$), and only He showed significant differences when using the whole SNP dataset (mean differences N vs S = - 0.0032; $P = 0.031$). Higher genetic diversity would be expected in lower latitude populations of the Northern Hemisphere because of their role as glacial refugia during Quaternary glaciation (Hewitt, 2000). Previous studies with microsatellite loci did not detect differences between both groups (Martínez et al., 2013), and even supported higher diversity in the Celtic/Irish Seas, English Channel and the North Sea (Martínez et al., 2015) corresponding to the present northern group. Moreover, higher genetic diversity has been found in northern beds from the Fennoscandian region and Russia, using mtDNA markers, which suggested a cryptic northern glacial refugia for *C. edule* (Krakau et al., 2012; Martínez et al., 2015), which has also been described for other marine species (Luttikhuizen et al., 2008; Maggs et al., 2008; Sotelo et al., 2020). Our extensive genome-wide analysis does not support consistent differences in genetic diversity for the edible cockle in the whole Northeast Atlantic evaluated, suggesting notable connectivity among samples.

5.2. Population structure and connectivity

Knowledge of population structure, including local adaptations, is crucial to define and apply sustainable strategies for the management and conservation of exploited species

(Frankham et al., 2002; Bernatchez et al., 2017). For *C. edule*, the presence of at least two main population units within its natural range, delimited by the western English Channel, had been reported in previous studies (Krakau et al., 2012; Martínez et al., 2015) and here has been confirmed with a more in depth population genomic analysis using the whole SNP dataset. The northern group in this study, comprising Celtic/Irish Sea, English Channel and North Sea beds, corresponds with the northern group defined by Martínez et al. (2015). This structure has also been documented in other mollusc species with a similar distribution range such as *O. edulis* (Vera et al., 2016). The results obtained for larval dispersal modelling based on potential ocean-driven larval flows between sites along the Northeast Atlantic by the University of Bangor partner within WP5, also suggests a major discontinuity at the French Brittany headland, indicating that the oceanography of the Northeast Atlantic plays an important role in shaping the genetic structure of cockles. The frontal systems (Ushant Front and Celtic Sea Front) when fully established seem to represent barriers to dispersal, thus limiting e.g. the transport of larvae into the English Channel and into the Irish Sea. Headlands, such as Brittany and Cornwall, also appear to act as barriers, potentially due to diverging current systems (Fig. 6).

Genomic scans aid detecting footprints of selection regarding the neutral background, which represents invaluable information for sustainable management and conservation of fisheries (Nielsen et al., 2009; Bernatchez, 2016). Genetic markers showing a significant departure, above or below the neutral dataset are potential outliers under divergent or stabilizing selection, respectively, which can unravel a fine-scale structure related to environmental variables critical for resources management (Vera et al., 2019; Coscia et al., 2020). Temperature, salinity and other abiotic factors have been suggested as potential drivers for adaptive differentiation in *C. edule* (Coscia et al., 2020), as in other marine species in the region (Vera et al., 2016; do Prado et al., 2018). In our study, the RDA analyses confirmed the effect of temperature in a wider geographical range, the major subdivision North-South in the Northeast Atlantic being associated with a latitudinal annual mean temperature gradient of ~5.5 ºC between the warmest (Ría de Formosa) and the coldest station (Dundalk).

Moreover, the analysis with outlier loci enabled us to disclose a significant substructure in the Northeast Atlantic beyond the two major groups identified with the whole dataset. Thus, the northern Atlantic group appeared also split into two major subgroups including the Celtic/Irish Sea beds in the west and the English Channel and North Sea beds in the east using outlier information. Concurrently, this northern subdivision was also detected with the larval dispersal modelling performed here. Within the southern group, Portuguese samples were subdivided in a southern group (Ría Formosa – PRF, Tejo Estuary – PTE_18, Sado Estuary – PSA_19) differentiated from the northern sample (Aveiro – PRA_17), which clustered with northwest Spanish beds (SNO, SLO). In the larval dispersal modelling within WP5, very weak connectivity was also observed between both Portuguese subgroups during June and July at depths of 15 m and 30 m, but more data on the spawning period are needed to confirm the role of ocean currents on the observed genetic subdivision (see e.g. Mahony et al., 2020). In fact, strong connectivities were detected during the other simulated months. Northwards, Cape Finisterre has been suggested as a biogeographical barrier for marine organisms (López-Jamar et al., 1992; Abaunza et al., 2008; Piñeira et al., 2008) including *C. edule*, separating northwest Spanish beds from those in the Bay of Biscay (Martínez et al., 2013). Microgegraphical analysis along Galician coast, which included beds at both sides of this discontinuity, did not find genetic differentiation at this region (global $F_{ST} = 0.0019$; $P > 0.05$), suggesting the presence of one panmictic unit and no effect of Cape Finisterre as biogeographical barrier for *C. edule* connectivity as described for other marine species (Domingues et al., 2010; López et al., 2015). The Arcachon bed (FAR_17) was significantly differentiated from the northwest Spanish group using outliers. However, this substructure did not appear to be related to dispersal limitations of larvae according to the modelling data provided by WP5 and more detailed sampling should be carried out in Biscay Bay to detect the possible barrier explaining the observed differentiations. Thus, and thanks to the results obtained in this study together with the collaboration of other COCKLES partners (i.e. UCC, UoB, UCaen, CIMA), new cockle beds along other biogeographical barriers and fronts identified in the AA for marine organisms (i.e. Celtic Sea Front, Cornwall Peninsula, tip of Brittany, Cotentin Peninsula) including fish (Abaunza et al., 2008; Larmuseau et al., 2009) and

molluscs (Dupont et al., 2007; Piñeira et al., 2008; Martínez et al., 2015; Nicolle et al., 2016; Handal et al., 2020) will be analysed to obtain a more precise picture of population structure. This refined analysis of genetic diversity at a local scale around the main fronts and physical barriers will provide essential information to understand the observed population structure patterns and their correspondence with larval dispersion and environmental drivers.

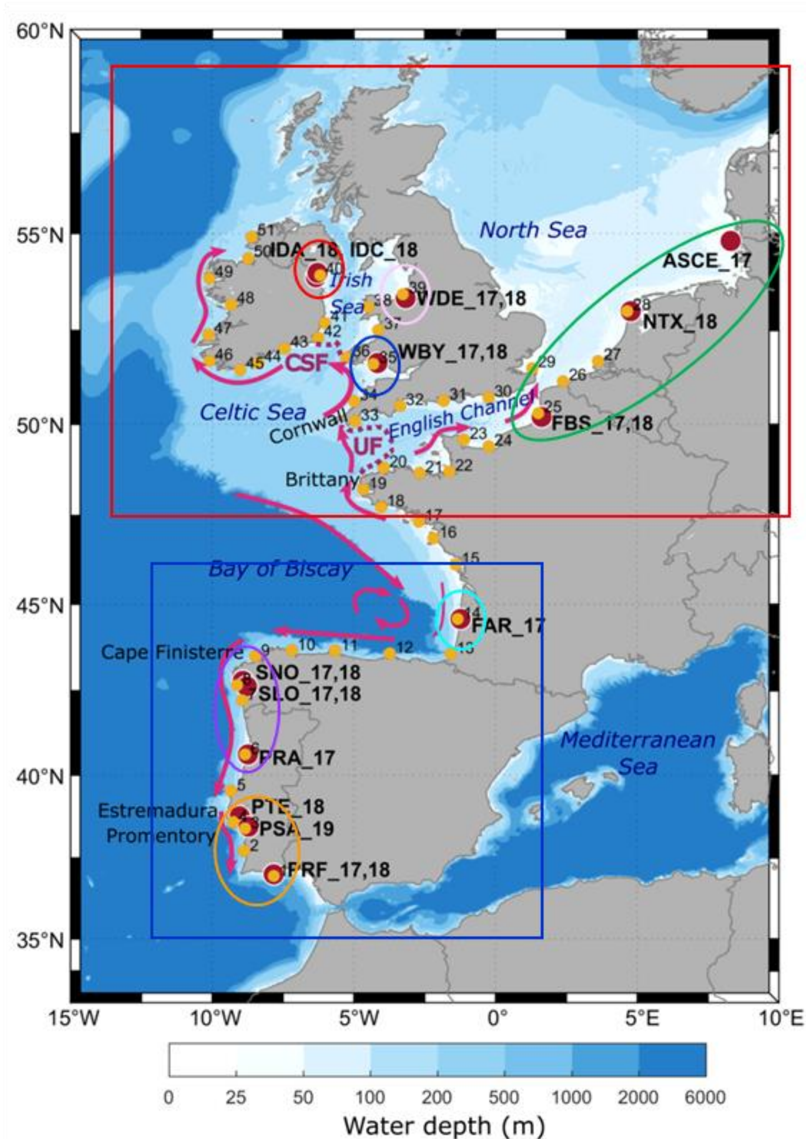


Figure 6. Study area for *C. edule* genetic analysis and larval dispersal modelling (in collaboration with UoB within WP5). Ocean bathymetry is shaded in blue. Summer surface currents are schematically represented by magenta-coloured arrows. Locations of oceanic fronts are depicted by purple dotted lines (CSF =Celtic Sea Front; UF =Ushant Front). Beds included for the genetic analysis are shown in dark red and particle release loctaions for larval dispersal modelling are shown in yellos and numbered from 1 to 51. Blue and red squares represent the

genetic groups for K =2 (all dataset and divergent outliers) while coloured circles represent the main seven genetic groups for K = 10 (using divergent outliers) obtained with fastSTRUCTURE (see Fig. 3). More detailed information about the geographical distribution of the different genetic groups identified in the AA is available on COCKLES GIS viewer ("Cockles genetics" layer at https://utmar.cetmar.org/cockles-viewer/). Bed codes are shown on Table 1.

5.3. Fisheries management

Our results represent useful information for the management of cockle beds in the Northeast Atlantic and could be valuable for obtaining suitable seed either for restocking of depleted populations or for finding broodstock to enhance cockle production. An improved definition of management units considering both demography and adaptation to environmental variation along the Northeast Atlantic can now be delineated, allowing the future definition of adaptive management units (AMU, Bernatchez et al., 2017). Two main operational units, located northwards and southwards French Brittany, can be defined as the basic proposal for management, but a more ambitious approach should include at least five different units: (i) Irish/Celtic Sea; (ii) English Channel/North Sea; (iii) Bay of Biscay; (iv) northwest Spain and north Portugal; and (v) south Portugal. Further, our data and those from Coscia et al. (2020) suggest that the Irish/Celtic Sea unit could be additionally refined. The information from this study might be useful to define sets of markers, starting from outlier loci, which could be applied to found brookstock for restocking depleted populations and to track individuals to their units that could aid the identification of illegal transferences between countries or from disease-affected areas. The obtained data within COCKLES project represents the baseline to monitor restocking and to evaluate the impact of intensive aquaculture on cockle beds. Further work will be done at a more refined scale combining genetic and larval advection patterns around the main areas of differentiation in the Northeast Atlantic suggested in this study.

6. Conclusions

The presence of two main genetic groups, northwards and southwards of French Brittany, was indubitable and was in accordance with previous genetic studies carried out with different molecular markers. Moreover, larval dispersal modelling developed in WP5 identified a barrier linked to the Ushant front. Further genetic subdivision was observed using outlier loci (under divergent selection) and considering larval behaviour (up to seven groups geographically distributed). Therefore, different operational units for management and conservation purposes (form two up to seven) were identified. Sea Surface Temperature (SST) could be an environmental driver explaining genetic differences (following a latitudinal axis). Refined microgeographic analyses along Galician coast suggested no influence of Cape Finisterre as biogeographical barrier, identifying a single panmictic for the assessed bed. Further studies along other barriers described previously in the AA will help to get a more comprehensive picture. The obtained information represents the baseline for management of cockles, allowing the design of conservation and management strategies, the foundation of broodstock for depleted beds, and the production of suitable seed for aquaculture production in order to allow the maintenance of this important natural resource.

7. Acknowledgements

L. Insua, S. Sánchez-Darriba and S. Gómez provided technical support. COCKLES, VIVALDI and Scuba Cancers partners, together with M.L. Conde-Varela, M.T. Fernández-Núñez, M. García-Graña, P. Luttikhuizen, S. Pereira, A. Simón, and L. Solís provided many of the samples analysed. Supercoputing Centers of Galicia (www.cesga.es) provided computing facilities and technical support for the genotyping and population genomics analyses.

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