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Original Article

Genetic divergence and adaptation of an isolated European lobster population in the Netherlands

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Abstract

Identifying isolated populations is a key step towards enacting effective conservation management. European lobsters (*Homarus gammarus*) from Oosterschelde in the Netherlands are subject to fishery pressure and have previously been reported as genetically differentiated. They are also putatively of transplanted origin and have subsequently endured recent bottlenecking and environmental change. We assessed Oosterschelde lobsters to evaluate their demographic independence and appraise potential founder effects and evolutionary responses to isolation. Using restriction-site associated DNA sequencing, we genotyped 6185 single nucleotide polymorphisms across 188 individuals from 27 sites across the Atlantic range of *H. gammarus* to investigate population genetic diversity, structure, and potential adaptation. Our results show that Oosterschelde lobsters are genetically divergent from other stocks. We evidence extensive differentiation via both neutral and outlier loci, indicative of strong biophysical and demographic isolation, and detect signatures of reduced genetic diversity that may reflect weak founder effects or subsequent population contractions. Among outlier loci, we identify candidates for range-wide local adaptation via variants in genes of important biological functionality and link a private allele of Oosterschelde to a locus potentially conveying adaptive tolerance to environmental hypoxia. Given our findings, we advise proactive monitoring of Oosterschelde lobsters to explore whether existing management measures effectively conserve this discrete, self-recruiting population.

Keywords: European lobster; zeekreeft; Oosterschelde; population structure; genetic diversity; gene flow; isolation; evolution; environmental adaptation; fisheries management

Introduction

The European lobster (*Homarus gammarus*; hereafter, 'lobster') is a large and long-lived decapod crustacean inhabiting shallow coastal seas in the northeast Atlantic and Mediterranean. The species is highly prized as a valuable seafood commodity, with recent global landings of ∼5000 tonnes per year, but several major stocks have suffered from collapse as a result of intensive fishing pressure and insufficient management (Kleiven et al. 2012, Ellis et al. 2015, Spanier et al. 2015). Within the Atlantic, the lobster ranges from Arctic Norway and the Kattegat to Morocco and shows spatial genetic structuring typified by a latitudinal cline of variation and a weak genetic break between Scandinavia and stocks to the southwest range-edge (Ellis et al. 2017, 2023, Jenkins et al. 2019). Alongside these broad patterns, isolated subpopulations showing genetic differentiation have been identified in northern Norway and in the Oosterschelde, Netherlands (Jørstad et al. 2004, 2005, Triantafyllidis et al. 2005, Jenkins et al. 2019). While lobsters in northern Norway appear isolated by the region's fjordal geography and the species' limits of environmental tolerance at its range-edge (Jørstad et al. 2005, Triantafyllidis et al. 2005), the factors driving divergence in Oosterschelde, which is nearer the centre of the species' range and its most productive fisheries (in eastern Britain), are less obvious. Indeed, given that lobsters prefer habitats of hard substrate that provide sheltering spaces, such as rocky reefs and boulder fields (Howard 1980), it is unclear why a sub-population should exist in the Oosterschelde at all, given that the basin's natural state is of a turbid estuary dominated by sand, silt, and mud seabeds (Louters et al. 1998).

Historically, there may not have been a lobster stock in the basin at all. Historic records state that there were no lobsters in the Oosterschelde, but that Dutch traders had begun importing live lobsters from Norway around 1650, and there are references to them being stored live in saltwater ponds constructed along the shore (Havinga 1921, Spanier et al. 2015, Verschuur et al. 2023). The ancestry of the Oosterschelde lobsters is attributed to the local shipwreck of a trade vessel in the late 17th century, whose cargo of live lobsters from Norway escaped (van Ysseldijk 1973), and/or to tidal floods or storm surges enabling escapes of such imported lobsters from holding ponds (Havinga 1921). In any case, it is thought that a considerable abundance of lobster (enough to warrant direct targeting by fishers) did not accrue until approximately 1900, when the local stock increased (Gmelig-Meyling and de Bruyne 2003, van Stralen and Smeur 2008). This growth coincided with increased construction of dykes around Oosterschelde, which may have improved conditions for lobsters via

the introduction of new rocky substrate (van Ysseldijk 1973, Verschuur et al. 2023), and the completion of a railway dam at Woensdrecht, which may have increased localized seawater retention (Havinga 1921). These factors likely helped establish a lobster fishery that yielded ~15–45 tonnes a year during the 1920s and early 1930s (van Stralen and Smeur 2008, Supplementary Material S1.1).

In attempts to protect low-lying land from persistent flood risk, the wider Rhine–Meuse–Scheldt delta area of the Netherlands' southwestern provinces then underwent a significant modification by construction projects in the latter half of the 20th century. This programme of construction, known as the Delta Works, consisted of a network of flood-gates, dams, and sluices to control and modify water flows, including two major dams separating the former Haringvliet, Grevelingen and Veerse Meer estuaries from the North Sea, and a system of man-made islands and retractable storm surge barriers across the mouth of the Oosterschelde that was completed in 1986 (Nienhuis and Smaal 1994, Louters et al. 1998) (Fig. 1b and c). These installations have had considerable effects on the geomorphology, biochemistry, hydrology, and ecology of the basin (Bakker et al. 1994, Louters et al. 1998), including its lobster stock. The winter of 1962/3 saw profound mass mortality of lobsters in the Oosterschelde, the apparent result of extremely low salinity and temperature caused by increased freshwater inputs that followed the disconnection of the Brouwershavense estuary by the Grevelingendam (Gmelig-Meyling and de Bruyne 2003, Triantafyllidis et al. 2005, Tangelder et al. 2012, Verschuur et al. 2023). It has been previously hypothesized that this event (as opposed to founder effects) caused a strong population bottleneck event, reducing genetic diversity and subsequently increasing genetic drift, resulting in the contemporary genetic differentiation of Oosterschelde lobsters (Triantafyllidis et al. 2005).

Nevertheless, a subsequent recovery of the stock observed in the 1990s fits a timeline of the completed works having shifted the basin's ecotype from a turbid estuary to a tidal bay (Louters et al. 1998). Salinity was raised and stabilized, generating a transition from estuarine assemblages towards fully marine taxa, thus improving local conditions for lobsters (Bakker et al. 1994, Gmelig-Meyling and de Bruyne 2003). Today, the main Oosterschelde sea-barrier gates are open in normal operation and water exchange with the North Sea remains, albeit at reduced levels (Nienhuis and Smaal 1994, Louters et al. 1998). Lobsters are now found in near-shore areas throughout much of the Oosterschelde, where abundance is most concentrated in areas of submerged peat bogs, wild oyster beds, and the rocky habitats provided by man-made foreshore protection (Gmelig-Meyling and de Bruyne 2003, Verschuur et al. 2023). Lobsters also inhabit the now enclosed Grevelingen and Veerse Meer, as a result of successful seeding with mature individuals from the Oosterschelde by local fishers in recent years (van Stralen and Smeur 2008). Currently, Oosterschelde lobsters are targeted in a licensed, seasonal fishery (March–July) by 24 small-scale fishers using passive gears (Verschuur et al. 2023). Landings in 2022 were estimated at around 31 tonnes and have been relatively stable over the past decade (Bleijenberg 2023, WMR 2023, Supplementary Materials S1.1).

Whereas previous studies have used mitochondrial DNA haplotypes (Triantafyllidis et al. 2005) and small panels of single nucleotide polymorphisms (SNPs) (Jenkins et al. 2019) to assert the genetic differentiation of Oosterschelde lobsters, our study aimed to use genome-wide SNPs derived from restriction-site associated DNA (RAD) sequencing to investigate the extent and potential drivers of genetic differences more thoroughly. Here, we address a series of questions relating to the potential genetic divergence and isolation of Oosterschelde lobsters: (i) the extent to which they diverge from other stocks in the species' Atlantic range; (ii) whether neutral and/or adaptive processes underpin their differentiation; (iii) whether genetic data supports a putative founder event in the Netherlands Delta area from lobsters of Scandinavian origin; and (iv) whether they should be managed as an isolated stock and meet established thresholds of genetic distinctiveness to be recognized as an evolutionary significant unit (ESU; Turbek et al. 2023). Accordingly, we hypothesized that the Oosterschelde lobster stock would be characterized by differentiation from most Atlantic stocks, have reduced genetic diversity and display ancestry signatures shared with Scandinavia.

Materials and methods

Sampling, DNA extraction and control, and RAD sequencing

Tissue samples from live lobsters were collected by collaborating fishers or researchers from 27 sites encompassing much of the species' Atlantic range (Fig. 1a; Table 1). Non-sacrificial tissue samples comprised small (~1 cm^2) sections of pleopod, uropod, or antenna excised from live individuals, and were stored at 4°C in individual tubes of >95% ethanol until sample processing. Genomic DNA was extracted from all tissues using a salting-out protocol (Jenkins et al. 2018). Purity of DNA was assessed via a Nanodrop 1000 spectrophotometer, and integrity and molecular weighting DNA fragments were evaluated using gel electrophoresis. DNA yields of extractions passing these checks were measured by the Promega QuantiFluor-ONE and GloMax Discover Systems, and concentrations standardized for sequencing. Exeter Sequencing Service, UK, undertook RAD library preparation using the restriction enzyme *sbfI*, for sequencing of 100 base pair (bp) (n = 48 lobsters processed in 2017) or 150 bp (n = 156 lobsters processed in 2019) paired-end reads (see Supplementary Materials S1.2 for full details of RAD protocols).

Generation of RAD loci and SNP calling

The raw read data from the two sequencing runs were amalgamated and trimmed of adapters and poly-G tails using `fastp` v0.20 (Chen et al. 2018), before being dephased. Reads were then cleaned, quality filtered, and truncated to 97 bp using the `Stacks` v2.54 (Catchen et al. 2011, 2013, Rochette et al. 2019) program process_radtags, following which samples with <300 000 retained reads were removed. The `Stacks` pipeline *denovo_map* was used to build RAD loci with optimized parameters for allowable stack mismatches within and between individuals (M/n = 2 for both species) to maximize SNP retention (Paris et al. 2017). The *populations* program of `Stacks` was run to isolate and process SNP genotypes from RAD loci, filtering globally for each species to retain only those SNPs that were present (i) in ≥90% of individuals, (ii) at ≤0.6 heterozygosity, (iii) at ≤0.01 MAF, (iv) in all 27 sampling locations, and (v) as the first variant on each RAD-tag (to help minimize linkage disequilibrium; Larson et al. 2014). To

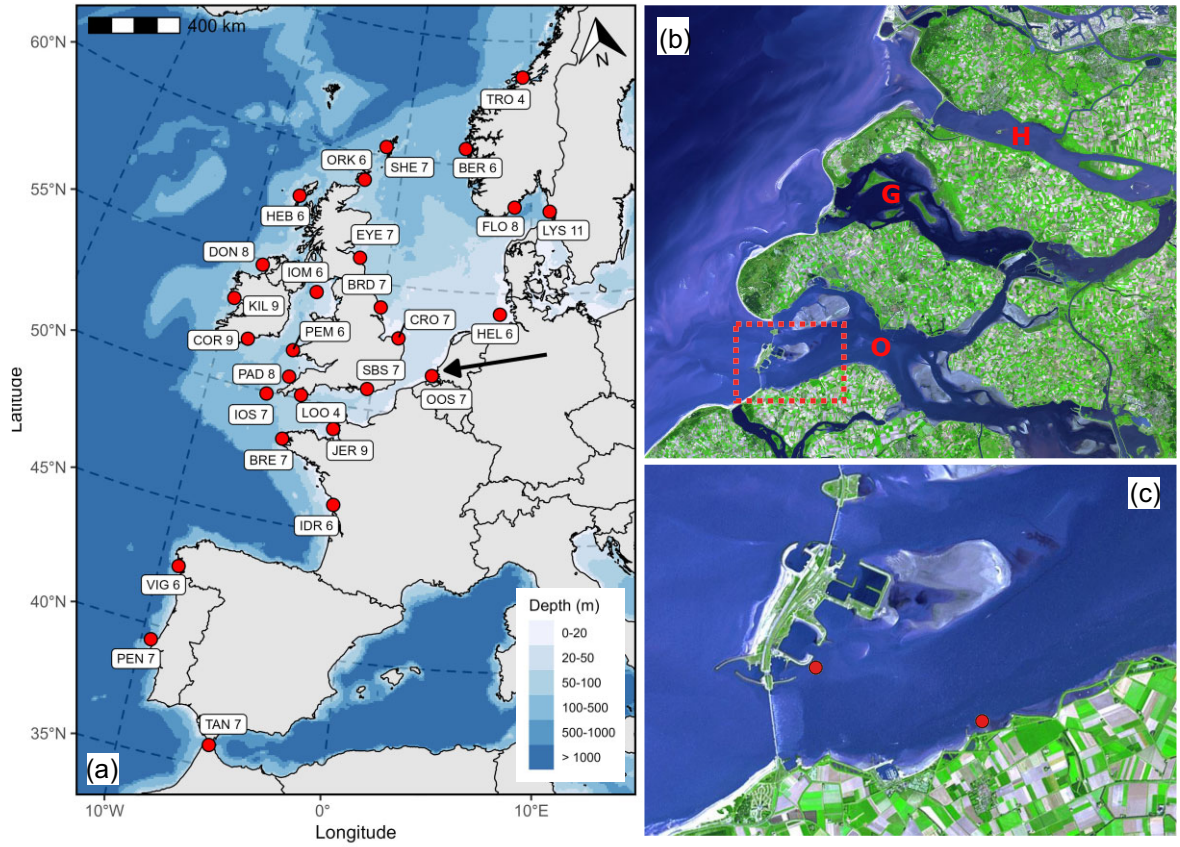

Figure 1 (a) Bathymetric map of western Europe with red circles showing the locations of sampling sites for lobsters Homarus gammarus, and labels denoting three-letter site codes and the number of individuals analysed (post-filtering). The arrow denotes the location of the Delta Works in SW Netherlands, the area shown in the satellite composite of (b), in which the main three inlets are (top to bottom) the Haringvliet (H), the Grevelingen (G), and the Oosterschelde (O). The dashed box highlights the area shown in (c), in which the capture locations of Oosterschelde lobsters used in this study are shown as circles, and the storm surge barrier is visible, demarking the boundary between the Oosterschelde (at right) and the North Sea (left). Images in (b) and (c) are adapted courtesy of the NASA Earth Observatory.

prevent the potential introduction of artificial structure via alternative protocols, no SNP filtering was conducted based on Hardy–Weinberg equilibrium (Pearman et al. 2022). Finally, using `R` v4.3.2 (R Core Team 2023), the *missingno* function of `poppr` v2.8.5 (Kamvar et al. 2014) was used to remove any individuals missing genotypes for ≥25% of these filtered SNPs, and the *isPoly* function of `adegenet` v2.1.10 (Jombart 2008, Jombart and Ahmed 2011) was used to remove loci subsequently rendered monomorphic.

Gene flow, diversity, and population structure

The R package `hierfstat` v0.04–22 (Goudet and Jombart 2015) was used to calculate sitewise expected and observed heterozygosity (H_e/H_o), inbreeding coefficient (F_{IS}), and allelic richness (A_R), as well as global and regional measures of differentiation as F_{ST} (θ; Weir and Cockerham 1984). Confidence intervals (95% CIs) for F_{ST} estimates were calculated via 100 bootstraps across loci in the R package `diveRsity` v1.9.90 (Keenan et al. 2013). To further probe sitewise variation in genetic diversity, the relationship between sample sizes and total observed polymorphisms per site ($Poly_o$) was characterized to enable the calculation of expected polymorphisms for a given sample size ($Poly_e$), and this then used to calculate the percentage differential between observed and expected polymorphisms ($d.Poly_{oe}$), as $d.Poly_{oe} = (100 - (Poly_o/Poly_e))$ × 100 (Supplementary Material S2). To further investigate potential signals of genetic isolation, the presence of private alleles was investigated using the *private_alleles* function of poppr, and the effective population size (N_e) was estimated for Oosterschelde and eight adjacent North Sea sites using `NeEstimator-v2` (Do et al. 2014), as operated via the *gl.LDNe* function of the R package `dartR` v2.9.7 (Gruber et al. 2018, Mijangos et al. 2022). To try and overcome the bias inherent in estimating N_e from small sample sizes (England et al. 2006, Marandel et al. 2020), we also estimated N_e for the same sites using the dataset of Jenkins et al. (2019), which contained far fewer loci but more individuals per site. As per the assumptions of this method, N_e estimations were based only upon neutral SNP loci, and were calculated with loci excluded based on minor allele frequency (MAF) thresholds of both 0.01 and 0.05 to compare results in case of inflationary effects by rare alleles (Marandel et al. 2020).

Genetic clustering and population structure were assessed via principal components analysis (PCA) using the *dudi.pca* function of the R package `ade4` v1.7–22 (Thioulouse et al. 2018). Sitewise estimates of pairwise F_{ST} were calculated in `hierfstat`, and the R package `marmap` v1.0.4 (Pante and Simon-Bouhet 2013) was used to calculate pairwise geographic distances between sites as minimum oceanic paths via the *lc.dist* function. Interrogation via the *getNOAA.bathy* and *get.depth* functions of marmap showed that some lobster cap-

Table 1. Summary table of sampling information and genetic diversity statistics across all SNP loci, where *n* denotes the number of individuals per site passing genotyping quality control; Region shows regional merging of undifferentiated samples for demographic analyses; Lat./Long. are approximate coordinates of origin; H_e/H_o denote expected/observed levels of heterozygosity; F_{IS} is the inbreeding coefficient; A_R is the allelic richness; $Poly_o$ is the total number of observed polymorphic loci per site, and; $d.Poly_{oe}$ is the percentage differential between $Poly_o$ and the expected number of polymorphic loci per site ($Poly_e$), with the latter calculated as a function of *n* as per the logarithmic relationship between *n* and $Poly_o$ across all sites. Information for Oosterschelde is highlighted with bold text.

Site name	*n*	Code	Region	Year	Lat.	Long.	H_e	H_o	F_{IS}	A_R	$Poly_o$	$d.Poly_{oe}$
Trondheim, Norway	4	Tro	NORW	2018	63.57	9.25	0.120	0.110	0.216	1.142[a]	2155	−8.23%
Bergen, Norway	6	Ber	NORW	2018	60.65	4.77	0.135	0.123	0.169	1.147	2814	−3.82%
Flødevigen, Norway	8	Flo	SKAG	2019	58.42	8.76	0.142	0.139	0.084	1.150	3347	0.34%
Lysekil, Sweden	11	Lys	SKAG	2017	58.26	11.38	0.146	0.138	0.095	1.151	3787	−0.06%
Helgoland, Germany	6	Hel	Hel	2017	54.18	7.90	0.134	0.134	0.089	1.146	2871	−1.88%
Oosterschelde, the Netherlands	**7**	**Oos**	**Oos**	**2017**	**51.61**	**3.72**	**0.131**	**0.126**	**0.112**	**1.141**	**2734**	**−13.08%**
Cromer, England	7	Cro	EAGB	2016	52.95	1.31	0.141	0.142	0.066	1.151	3244	3.13%
Bridlington, England	7	Brd	EAGB	2017	54.07	−0.12	0.143	0.147	0.048	1.153	3310	5.23%
Eyemouth, Scotland	7	Eye	EAGB	2017	55.88	−2.06	0.139	0.135	0.107	1.149	3082	−2.02%
Shetland, Scotland	7	She	SCOT	2017	60.12	−1.43	0.143	0.147	0.047	1.152	3264	3.77%
Orkney, Scotland	6	Ork	SCOT	2017	59.01	−2.78	0.135	0.134	0.094	1.148	2910	−0.54%
Hebrides, Scotland	6	Heb	SCOT	2017	57.79	−7.25	0.133	0.132	0.097	1.145	2853	−2.49%
Donegal, Ireland	8	Don	EIRE	2016	54.93	−8.55	0.146	0.152	0.029	1.155	3498	4.87%
Kilkieran, Ireland	9	Kil	EIRE	2016	53.25	−9.80	0.143	0.135	0.116	1.151	3264	−6.83%
Cork, Ireland	9	Cor	EIRE	2016	51.66	−8.42	0.145	0.140	0.087	1.152	3570	1.90%
Peel, Isle of Man	6	Iom	IRIS	2016	54.21	−4.75	0.140	0.142	0.069	1.152	3048	4.17%
Pembrokeshire, Wales	6	Pem	IRIS	2016	51.81	−5.29	0.140	0.143	0.063	1.152	2994	2.33%
Padstow, England	8	Pad	CORN	2017	50.60	−4.93	0.142	0.144	0.052	1.151	3352	0.49%
Looe, England	4	Loo	CORN	2016	50.29	−4.30	0.135	0.147	0.053	1.154	2546	8.42%
Isles of Scilly, England	7	Ios	CORN	2016	49.90	−6.36	0.141	0.143	0.065	1.151	3172	0.84%
Shoreham, England	7	Sbs	CHAN	2016	50.81	−0.26	0.142	0.141	0.082	1.153	3226	2.56%
Jersey, Channel Isles	9	Jer	CHAN	2016	49.05	−1.95	0.143	0.146	0.039	1.151	3600	2.76%
Brest, France	7	Bre	FRAN	2019	48.31	−4.84	0.138	0.132	0.112	1.148	3135	−0.33%
Île de Ré, France	6	Idr	FRAN	2017	46.09	−1.27	0.138	0.141	0.060	1.150	2999	2.50%
Vigo, Spain	6	Vig	IBER	2017	42.49	−8.99	0.138	0.128	0.156	1.150	2936	0.35%
Peniche, Portugal	7	Pen	IBER	2019	39.41	−9.51	0.137	0.134	0.095	1.147	3042	−3.29%
Tangiers, Morocco	7	Tan	Tan	2019	35.84	−5.63	0.138	0.134	0.104	1.148	3113	−1.03%
						Mean	*0.139*	*0.137*	*0.089*	*1.150*	*3142*	*N/A*

[a]= Assessed across only 6181 SNPs, after removal of four loci missing genotype data for all individuals.

ture locations were in shallow waters (<2 m), but when run to accommodate this minimum depth, *lc.dist* inferred pairwise paths that occasionally traversed low landmasses or other impenetrable barriers. As such, to ensure that minimum paths between sites were fully oceanic, some site co-ordinates were adjusted up to ∼2 km to ensure depth was negative and normalized at 10–120 m. Based on clustering results and pairwise F_{ST} estimates between sampling locations, samples from adjacent sites that showed minimal divergence were pooled to increase sample sizes and the robustness of subsequent demographic analyses. Pairs or triplets of sampling sites that had pairwise $F_{ST} < 0.01$ across all loci and which were <485 km apart and were merged, giving them sample sizes of at least $n = 10$ (Supplementary Material S3.1/S3.2 for all site geographic/all loci genetic distances; Table 1 for resultant sample pooling information). Only Oosterschelde (Oos) and the sites of Helgoland (Hel) and Tangiers (Tan), for which the nearest adjacent samples were ≥485 km away, remained separate. Coordinates for pooled samples were retaken as the central oceanic point between sampling locations, and these used to recalculate pairwise minimum paths between them (Supplementary Material S3.3). Mantel tests between matrices of pairwise genetic and geographic distance (F_{ST} and km, respectively) assessed isolation-by-distance (IBD), using the *mantel.rtest* function of `ade4`, with *P*-values estimated across 10 000 Markov Chain Monte Carlo permutations. Following sample merging of neutral genotype data, Mantel tests were unable to directly assess IBD solely for Oosterschelde comparisons, due to the number of comparisons (12) being incompatible with matrix formatting. To overcome this, six replicates of matrices each containing 10 comparisons were created, with two randomly selected comparisons omitted from each replicate (Supplementary Material S3.6).

The optimal number of neutral genetic clusters (*K*) and individual membership of these were also explored using `adegenet`; the *snapclust.choose.k* function enabled comparisons of the corrected Akaike information criterion (AICc) across each modelled value of *K*, and the *snapclust* function performed maximum-likelihood estimations of individual assignment probability to each cluster at given values of *K*, both of which are instructive in identifying the likely value of *K* sampled (Beugin et al. 2018). To explore potential shared ancestry, for sites within 1500 km of Oosterschelde (i.e. all those except Iberian and Moroccan samples), the results of primary cluster assignments at an optimized value of global *K* were displayed on an equal area map of the study area using the R package `mapmixture` v1.1.0 (Jenkins 2024). Other key results were visualized using the R package `ggplot2` v3.4.4 (Wickham 2016).

Selection and genome alignments

Two methods were applied to detect outlier SNPs potentially under selection; the Bayesian differentiation-based method of `Bayescan` v2.1 (Foll and Gaggiotti 2008) and the likelihood-based F_{ST} distribution model of `OutFLANK` v0.2 (Whitlock

and Lotterhos 2015). To minimize the possibility of false positives in the detection of SNP outliers, the expectation that sitewise variations in allele frequencies arose from neutral demographic effects was considered 10 000x more likely than effects of selection for `Bayescan`, and the False Discovery Rate was controlled equivalent to $P < .05$ (Benjamini and Hochberg 1995). For `OutFLANK`, the alpha parameter for was adjusted to $P < .01$, which also helped to yield outlier subsets of similar sizes between methods. For each method, we ran outlier detection with two distinct sampling priors: (i) as separate fine-scale sites ($K = 27$), and; (ii) with Oos individuals segregated from an Atlantic meta-population featuring all other individuals ($K = 2$).

RAD-seq contig sequences corresponding to the identified outlier loci were aligned to the reference genome of the European lobster (GCA_958450375.1) using `minimap2` (Li 2018), retaining only those loci with the maximum quality score (MAPQ = 60), which denotes mapping to a single unique genomic region. The alignment file was converted to BED format using the *bam2bed* function of `BEDOPS` v2.4.35 (Neph et al. 2012) and intersected with the GTF annotation file with the *intersect* tool in `bedtools` v2.29 (Quinlan and Hall 2010). For the contigs which aligned to exon gene features, we translated the gene ID to coding sequence (CDS) using `AGAT` v0.9.1 (Dainat 2020). The translated CDS was used as an input to *BlastP* (Altschul et al. 1990) to obtain the gene name via homology searching against other decapod species. To predict the effects of each variant locus in gene-coding regions, we used *stacks-integrate-alignments* (Rivera-Colón and Catchen 2022) using the alignment file to liftover the SNP positions to the genome and used `SnpEff` v.5.2 to predict the functional effects of individual SNPs (Cingolani et al. 2012).

Results

Sequencing depth and SNP discovery

Of the 204 lobster samples sequenced, one was removed prior to bioinformatic processing due to an insufficient number of raw reads. The subsequent RAD catalogue was built using 203 individuals, across which the mean number of RAD-tags was 34 313, and the mean sequencing depth was 44x coverage. Global quality-control SNP filtering yielded 9812 variant loci, though upon obtaining individualized haplotype data, a further 15 individuals were removed from the analysis due to missing genotypes at ≥25% loci. Following this removal of samples failing missing data thresholds, some 3627 loci were rendered monomorphic; their removal left a final dataset featuring 188 lobsters genotyped across 6185 polymorphic SNPs.

Genetic diversity and differentiation

Among sampling locations, H_e ranged from 0.120 to 0.146 with a mean of 0.139, and H_o from 0.110 to 0.152 with a mean of 0.137, with $H_o = H_e \pm 0.012$ in all sites (Table 1). Mean sitewise F_{IS} was 0.089 and ranged from 0.029 to 0.216, being lowest in Donegal and highest in Trondheim, while mean sitewise A_R was 1.150 and ranged from 1.141 to 1.155, lowest in Oosterschelde and highest in Donegal. Sitewise $Poly_o$ varied from 2155 (Tro, $n = 4$) to 3787 (Lys, $n = 11$). Though direct comparisons of these raw values are biased by uneven sample sizes, this is overcome by comparing observed ($Poly_o$) with expected polymorphisms ($Poly_e$) as a function of sample size (Supplementary Material S2). A logarithmic relationship ($r^2 = 0.84$) between the number of individuals sampled (n) and $Poly_o$ established $Poly_e = (\ln(n) \times 1424.4) + 373.7$, from which the calculated $d.Poly_{oe}$ varied from −13.08% in Oosterschelde to +8.42% in Looe (Table 1). Across all samples and loci, F_{ST} was 0.008 (95% CIs 0.007–0.009), F_{IT} was 0.101 and F_{IS} was 0.094. Three loci were flagged as having private alleles, all of which were unique to Oosterschelde lobsters. Estimated N_e based on our neutral SNPs were infinite for all sites investigated, regardless of the MAF threshold used (Supplementary Material S4). When based on the 71 neutral loci and larger sample sizes of Jenkins et al. (2019), N_e estimates were infinite in three sites (Ber, Flo, and Lys) and had upper confidence intervals of infinity for the rest. Several sites had a lower estimated N_e than Oosterschelde, with Eyemouth recording the lowest (Eye N_e = 39.0–39.2; CIs 13.3–Inf.).

Of the total 78 pairwise F_{ST} estimates made using neutral loci following merging of sampling areas, two negative values (both $F_{ST} = -0.001$) were converted to zero. Pairwise F_{ST} was highest when area pairs included Oosterschelde (pairwise F_{ST} = 0.030–0.050, mean = 0.037; min. F_{ST} = Oos vs NORW, max. = F_{ST} Oos vs Tan). All 12 pairwise comparisons featuring Oosterschelde yielded $F_{ST} > 0.03$, compared to a maximum F_{ST} of 0.023 (Tan vs Hel) among the 66 comparisons without Oosterschelde; beyond that, $F_{ST} < 0.015$ for all comparisons (Fig. 2a; Supplementary Material S3.4). When grouping all other samples together as a single Atlantic unit run against Oosterschelde, global $F_{ST} = 0.035$ (Atlantic n = 181 vs Oos $n = 7$). Including all 78 pairwise area comparisons, Mantel testing showed no significant association between geographic and genetic distance ($P = .28$), for which the correlation coefficient was very low ($r^2 = 0.005$). However, this lack of overall IBD was caused by a clear split in clusters between pairwise comparisons made with and without Oosterschelde (Fig. 2b). Excluding Oosterschelde data ('Pairs without Oosterschelde'; Fig. 2b), there was highly significant positive IBD among remaining pairwise comparisons ($P < .001$, $r^2 = 0.41$). For Oos pairs, positive IBD ($r^2 = 0.35$) was significant ($P < .05$) in three of the six replicates, but the mean of all six was $P = .10$ (Supplementary Material S3.5 and S3.6).

Outlier detection and genetic structure

When assessing the Oosterschelde site against all other individuals as a single Atlantic sample group, neither Bayescan nor OutFLANK detected any outliers. Across all sites, as sampled, Bayescan identified 28 SNPs and OutFLANK 21 SNPs as outlier candidates potentially under selection. Eighteen of these outliers were common to both methods of detection, resulting in a subset of 31 outlier candidates identified by one or both methods, with the most divergent locus showing a global $F_{ST} > 0.2$. All three loci with private alleles for Oosterschelde were detected in the outlier analysis, as were three loci considered as near-private for Oosterschelde, in which more minor alleles were present in the Oosterschelde sample than all other samples combined (Supplementary Material S5). Removal of the 31 outlier loci resulted in a dataset of 6154 neutral SNPs. Using these 6154 neutral SNP loci (global $F_{ST} = 0.007$; 95% CIs 0.006–0.008), PCA strongly separated all Oosterschelde individuals from the remaining samples along the most powerful PC axis (which encompassed 1.0% of the total variation) (Fig. 3a). Via both axes 1 and 2 (0.8% of the variance), Scandinavian samples clustered away from the remaining Atlantic samples, although there was a small amount of over-

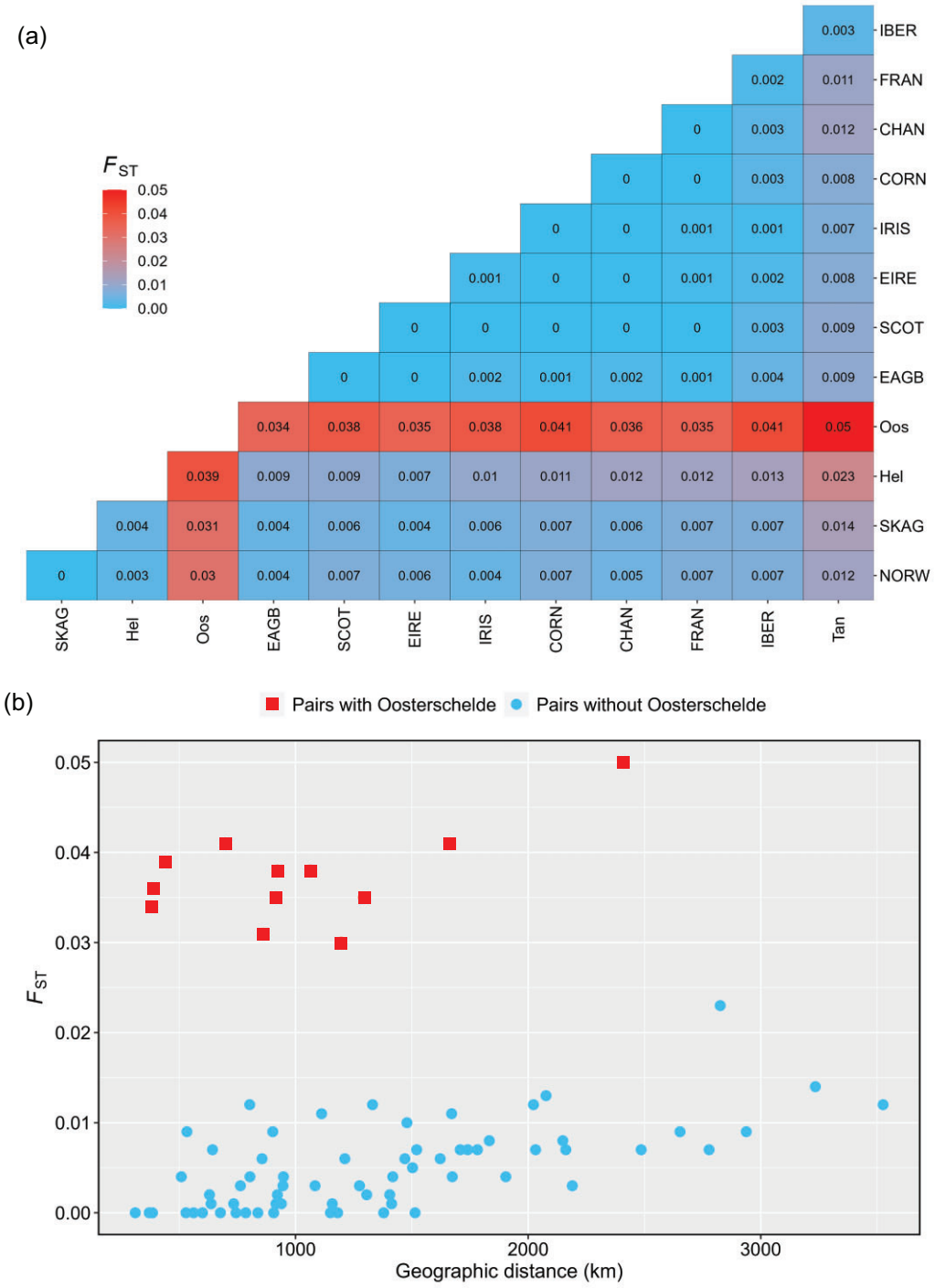

Figure 2 (a) Heatmap denoting pairwise FST between sites/areas, coloured as per the FST scale, and (b) isolation by distance, plotting genetic (FST) against geographic distance (km; minimum oceanic paths) between pairwise sites/areas. In (b), square points denote pairwise comparisons featuring Oosterschelde with other sites/areas, while round points denote non-Oosterschelde sites/areas paired with each other. Both analyses used genotype data across only the 6154 neutral SNP loci.

lap between these clusters at an individual level. Using the 31 outlier loci (global $F_{ST} = 0.239$; 95% CIs 0.208–0.270), PCA axes 1 and 2 produced a spatial genetic signature that clearly depicted a latitudinal cline driven by sample means, although there was considerable individual-level variation (Fig. 3b). The Moroccan sample (Tan) was outlying on axis 1 (36.6% of the variance), and there was sufficient structuring of sample means to split the Atlantic clade into three groups, with additional sub-clusters representing Iberia (Vig and Pen; positioned between a Western Britain and France group and the Moroccan sample), and Eastern Britain (Eye, Brd, and Cro; positioned between the Western Britain and France and Scandinavian clusters). Oosterschelde individuals typically aligned with the Scandinavian cluster via axis 1, but were mostly differentiated from them, and all other samples, along axis 2 (13.4% of the variance).

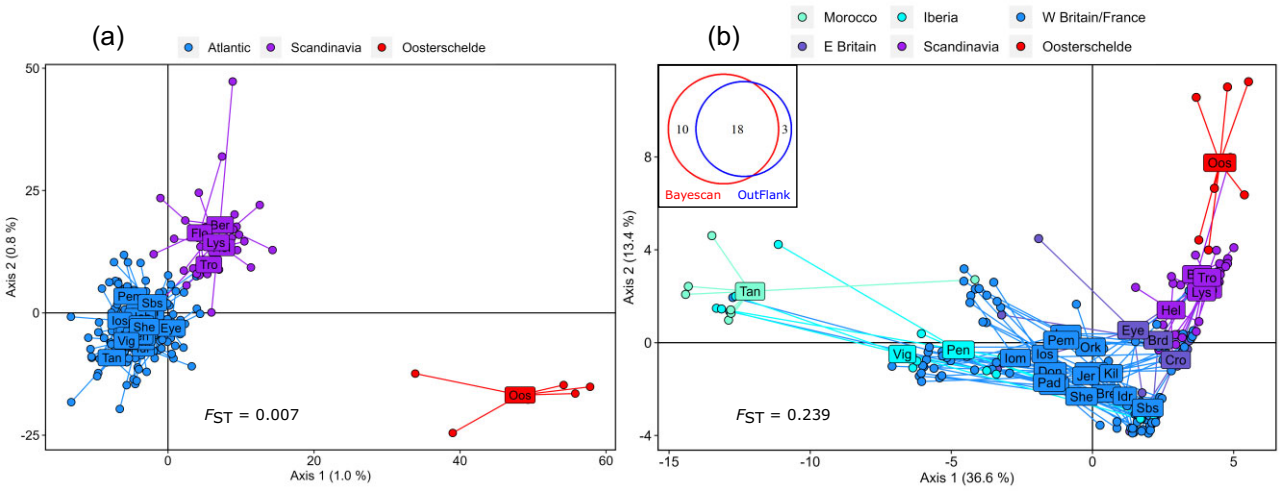

Figure 3 (a) PCA of 6154 neutral SNPs, and (b) PCA of 31 outlier SNPs, with corresponding FST shown across all samples. Round points denote the position of individuals, and labels the position of the sample site mean, both of which are coloured according to the resulting pattern of regional structure, as per the legend at top. The percentage of overall genetic variation explained by each axis in each analysis shown on axis labels. The Venn diagram inset in (b) shows the number of outliers identified by each detection method.

Using the 6154 neutral SNP loci and with sampling sites merged where appropriate, assessment of snapclust models run with continuous values of K showed that the largest drop in AICc occurred at $K = 2$, although AICc continued to drop with all additional values of K up to the number of spatial samples (Fig. 4b). Plateauing of the reduction in AICc with increased K hinted at the true number of clusters being $K \approx 6$, but inspection of genetic cluster memberships only supported spatial structuring up to $K = 3$, beyond which additional clusters further fragmented established Atlantic clusters or contained only single individuals. When run with $K = 3$, snapclust produced a cluster to which assignment was solely by all Oosterschelde individuals, and two clusters that loosely fit a spatial pattern of characterizing the established latitudinal cline of variation across the NE Atlantic, albeit with within-sample variation of primary assignments at an individual level; only the samples from Helgoland, Germany (Hel), and Western Norway (NORW—Ber and Tro) were not mixed, with all individuals assigning to the Atlantic cluster more prevalent among northerly locations (Fig. 4a; Supplementary Material S6.1). When run with $K = 2$, snapclust assigned all Oosterschelde individuals to one cluster and all remaining individuals to the other, and upon activating the hybrid functionality of snapclust, the algorithm detected no individuals of mixed ancestry (Supplementary Material S6.2). The integrity of the Oosterschelde cluster was extremely strong; while all other sites and areas experienced mixed assignment to multiple clusters by $K = 5$, all Oosterschelde individuals assigned to a single discrete clusters until K was inflated to >20.

Alignment of outlier loci to the genome and identification of candidate loci

Thirty of the 31 outlier loci aligned with maximum mapping quality to a unique region of the European lobster reference genome. Intersecting the alignments with the annotation showed that 18 loci aligned to intergenic regions and 13 aligned to gene regions. Of the 13 that aligned to gene regions, 11 were intronic, and six aligned to exon features, including a 5′ untranslated region (UTR) and a 3′ UTR. High homology between the translated CDS sequence and other Decapod proteins was found (E-value = 0.0; % identity ≥ 99%). Identified genes included: fructose-2,6-bisphosphatase TIGAR-like (TIGAR); sodium channel protein Nach-like; discoidin domain-containing receptor 2-like (DDR2); polyamine-transporting ATPase 13A3-like (ATP13A3); Kruppel-like factor 18-like (KLF18); peptidyl-prolyl *cis–trans* isomerase-like 3 (PPIL3). SNP variants predicted to induce functional changes upon transcription were found for TIGAR (missense mutation), ATP13A3 (missense mutation), and KLF18 (missense mutation and splice gene variant) (Supplementary Material S5). We discuss the effects of these variants and the relevant biological functionality of identified genes below.

Discussion

Our results show that lobsters inhabiting the Oosterschelde are clearly and distinctly genetically divergent and isolated from other surrounding stocks, as the primary feature of spatial genomic structure across the species' Atlantic range. Across multiple methods, we show that both neutral and outlier loci contribute to this divergence, suggesting that both demographic isolation and environmental adaptation may underpin the observed genetic differentiation of Oosterschelde lobsters.

Genetic diversity and founder effects

We found only tentative signals indicative of lower genetic diversity in Oosterschelde lobsters, as would be expected from strong founder effects and/or an acute population bottleneck. Both observed and expected heterozygosity were below average, while F_{IS} was above the mean, but all were within the normal ranges of values for other sites sampled across the Atlantic. Moreover, estimated N_e was high and comparable to other sample sites. However, individuals sampled from Oosterschelde did have the lowest allelic richness across all the sites we investigated (Oos $A_R = 1.141$; all other sites $A_R = 1.145$–1.155), and the number of polymorphic loci observed ($Poly_o$)

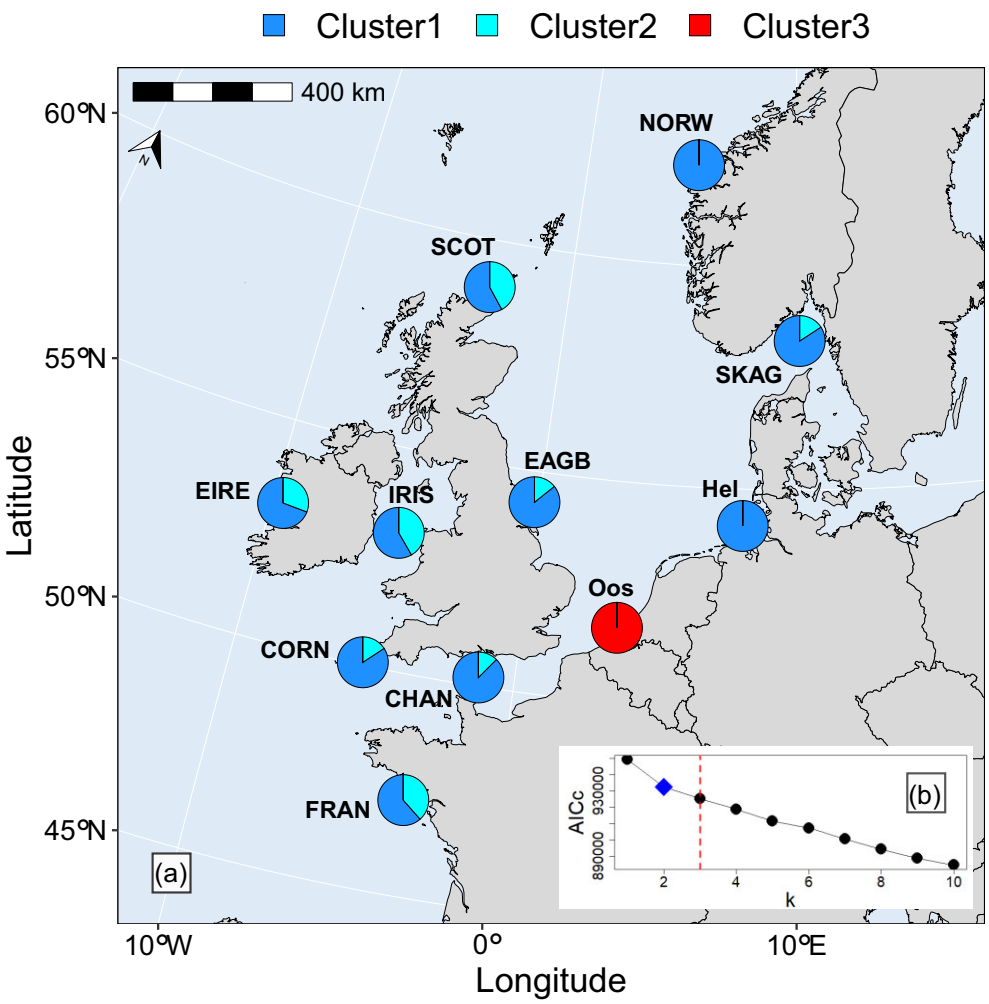

Figure 4 (a) An equal area project map of northwestern Europe with pie charts showing primary membership to each of three genetic clusters identified by snapclust using the 6154 neutral SNPs, with segment sizes proportional to the assignment of individuals in each sampling location or pooled area, and coloured as per the legend at top. Inset (b) shows corrected AICc scores for each value of total population clusters (*K*) modelled by snapclust. In (b), the diamond point marks the sharpest plateauing of AICc, $K = 2$, using which primary assignment membership simply segregated Oosterschelde individuals from all others, though spatially relevant cluster memberships were obtained up to $K = 3$, as denoted via the dashed line, and depicted in (a).

in Oosterschelde was 13.08% lower than the number of polymorphisms expected ($Poly_e$) based on its sample size relative to the relationship across all sites. No other site deviated from the mean relationship by as much as 8.5%, and Oosterschelde's outlier status as the most polymorphism-deficient site was highlighted by the overall fit (R^2) of the logarithmic relationship between sample size and SNP discovery, which rose from 0.845 to 0.899 when Oosterschelde samples were excluded (Supplementary Material S2). These small but conspicuous deficiencies in important measures of genetic diversity may be a remnant of a founder effect or bottleneck, which can lower allelic richness even where overall heterozygosity is maintained (Greenbaum et al. 2014). However, if these decreases do convey a founder effect, then it is relatively modest (Dlugosch and Parker 2008).

How then have Oosterschelde lobsters managed to largely maintain genetic variation despite the strong isolation signalled via their extensive differentiation? It may be that the original founding stock was sufficiently large and genetically diverse to prevent significant subsequent allelic loss or that multiple founder events have occurred (Dlugosch and Parker 2008). Similarly, occasional migration may have generated just enough gene flow to maintain diversity at levels close to those in surrounding stocks. Only two or three migrants per generation are generally required to maintain equilibrium in most allele frequencies (Lowe and Allendorf 2010, Greenbaum et al. 2014), and very rare long-distance dispersal events are sufficient to prevent considerable genetic differentiation or diversity loss, even in sessile marine taxa (Macleod et al. 2024). This may occur even without the most obvious potential mechanism of incoming gene flow; the natural settlement of larvae from external stocks in adjacent regions. Several American lobsters (*Homarus americanus*) have been recovered by local fishers in recent years, presumably escaped from shore-side merchants or released alive by members of the public. A mechanism of divergence resulting from hybridization with *H. americanus* can be ruled out on the basis that a sample of 40 Oosterschelde individuals recently tested, including all seven analysed in this study, showed no evidence of interspecific introgression (Ellis et al. 2020). However, local merchants also import live *H. gammarus*, principally from UK markets, so it is plausible that some may have been introduced to the Oosterschelde in the same way and have bred with local lobsters. As such, it is possible that the occasional estab-

lishment of incoming migrants from anthropogenic sources (and/or sporadic natural recruitment) has generated enough gene flow to mitigate genetic drift sufficiently to prevent the total loss of much allelic diversity, but not enough gene flow to prevent considerable drift in some allele frequencies or the accumulation of adaptive variation (Lowe and Allendorf).

Even without any migrants, it is possible that strong selection pressures from a novel environment with conditions at or close to limits of the species' tolerance (i.e. dissolved oxygen, salinity, temperature) could trigger sufficient accumulation of mutations to mitigate the effects of genetic drift in the wake of a founder event or other population bottleneck (Dlugosch and Parker 2008, de Bruyn et al. 2014). We did find private and near-private alleles in Oosterschelde lobsters, although it is debatable whether novel variants could sufficiently offset genetic diversity loss if an acute bottlenecking event occurred in combination with physical isolation, particularly over a time frame of only decades to centuries. Sparks et al. (2023) showed that genomic divergence of recently transplanted pink salmon (*Oncorhynchus gorbuscha*) was caused by rapid adaptive variation rather than genetic drift, but there was still a strong signal of overall genetic diversity loss (polymorphic loci <37.7%, H_o < 8.2%) from founding effects. In Oosterschelde, if migrants are typically rare, but prior founder effects or bottlenecks were severe, it is possible that genetic diversity may have been quickly rebuilt during subsequent population growth (Gmelig-Meyling and de Bruyne 2003, de Bruyn et al. 2014). Yet it is also quite possible that demographic contractions in Oosterschelde lobsters never generated severe genetic bottlenecks in the first place. Hailer et al. (2006) found that European populations of white-tailed eagle (*Haliaeetus albicilla*) were subject to considerable demographic bottlenecks and demonstrated very limited dispersal between isolated strongholds, yet genetic diversity loss among them was minimal, having been mitigated by the buffering effect of the birds' longevity. A more recent study of connectivity in the pink sea fan (*Eunicella verrucosa*), a long-lived marine invertebrate, reported similarly limited losses of genetic diversity even among isolated populations subject to depleted abundance (Macleod et al. 2024). European lobsters can also have relatively long lifespans (e.g. ~31–54 years; Sheehy et al. 1999), so it may be that most genetic diversity has been adequately maintained over what are short-term demographic constrictions relative to the lengthy generation time of lobsters.

Genetic differentiation in oosterschelde lobsters

Oosterschelde lobsters were found to be highly differentiated from other Atlantic lobsters and were far more differentiated from other sites than they were to each other. Globally, F_{ST} across all loci, outliers included, was only 0.008, but pairwise values featuring Oosterschelde averaged 0.037 even with neutral loci. Excluding their comparisons with Oosterschelde, no other site or area had a mean neutral pairwise differentiation exceeding $F_{ST} = 0.01$ (attained by both Tan and Hel), and Oosterschelde was more differentiated to all other sites and areas than any of them were to each other (Oos pairs, all $F_{ST} > 0.03$; others max. = Tan vs Hel, $F_{ST} = 0.23$). Indeed, sites geographically adjacent to Oosterschelde were far more differentiated to it than they were to other far more distant areas. The Eastern British (EAGB) and English Channel (CHAN) sample areas are at least four times closer to Oosterschelde than they are to the Iberian sample area (IBER) yet are around ten times more genetically distinct from Oosterschelde. Similarly, both EAGB and CHAN samples are around three times more differentiated from Oosterschelde, less than 400 km away, than the two most disparate samples in our analysis are from each other (NORW to Tan, 3526 km), despite the latter being at least nine times further apart in seaward distance (Supplementary Material S3.3 and S3.4). Indeed, the measure of overall differentiation we report between our Oosterschelde sample and the remaining 181 Atlantic lobsters ($F_{ST} = 0.035$) is even higher than that reported between these same Atlantic samples and 33 individuals of Mediterranean origin ($F_{ST} = 0.031$), despite the latter characterizing an established phylogenetic break (Ellis et al. 2023).

The differentiation of Oosterschelde lobsters was further evident via PCA. With neutral SNPs, the dissimilarity of all Oosterschelde individuals to those of other sites was conspicuous. While the established patterns of Scandinavian differentiation and a latitudinal cline in the Atlantic were characterized by adjacent clusters and a gradient of sample means rather than individuals, the Oosterschelde sample was entirely separated and by far the clearest element of spatial genetic divergence (Fig. 3a). Outlier SNPs also strongly differentiated most individuals and the Oosterschelde sample mean away from others via PCA (Fig. 3b). Using neutral loci and merging undifferentiated sites to sampling areas, snapclust assigned membership to inferred ancestral clusters in which all Oosterschelde individuals occupied a single discrete cluster at all plausible values of *K*, and there was no value of *K* at which an Oosterschelde individual shared membership of a cluster with any individual from another site. Similarly, although global IBD was strongly significant when Oosterschelde comparisons were omitted, there was only very tentative evidence for IBD among Oosterschelde comparisons assessed separately, with half of the comparison replicates tested failing to demonstrate a significant association between genetic and geographic distance (Supplementary Material S3.5 and S3.6). This break from the wider pattern of IBD is in itself a signal of Oosterschelde differentiation, yet even if we consider these results as a signal of weak IBD, such a finding would not be incompatible with strong genetic divergence. Assuming a relatively recent founder event for the Oosterschelde lobster stock, weak IBD may be an artefact of prior distance-based restrictions on gene flow before a more profound recent isolation, which would increase the relative sitewise differentiation via accelerated genetic drift (Ravinet et al. 2017). Alternatively, it may simply reflect that whatever low levels of genetic exchange have occurred more recently continue to be more likely to arise with adjacent stocks than distant stocks.

Demographic inferences

How demographically isolated is the Oosterschelde lobster stock in relation to others in the northeast Atlantic, and what long-term effect might the Delta Works have on their population? Even during the construction of the storm surge barrier, the Oosterschelde has not been physically isolated from the North Sea in modern times, and yet the levels of divergence we demonstrate in Oosterschelde lobsters indicate that migration into the basin has been very low or even absent (Lowe and Allendorf 2010, Greenbaum et al. 2014). The continental coast for at least 100 km either side of Oosterschelde is dominated by relatively featureless sandy seabeds, which make poor lob-

ster habitat (Howard 1980), so it is likely that incoming seawater to Oosterschelde has seldom contained lobster larvae beyond extremely low densities. The installation of the Oosterschelde storm surge barrier has cut tidal velocity by 20%–40%, tidal range by 12%, and tidal volume by 30% (Louters et al. 1998). While this now makes it even more unlikely that larvae from external sources enter the basin, it also implies that fewer native larvae are lost to the North Sea than occurred prior to the barrier's construction. This may mean more larvae are retained within the basin long enough to complete their planktonic life stages, improving local settlement and recruitment (Havinga 1921, Verschuur et al. 2023). Our Oosterschelde sampling occurred in recent years, and without a comparable temporal sample taken prior to the Delta Works, it is impossible to speculate how the changed conditions have influenced the population genetics of Oosterschelde lobsters, but this would be an interesting avenue for further study over the coming decades. The extent of neutral variation we observe supports Oosterschelde lobsters being recognized as a discrete management unit, while the extensive divergence of the population supports its recognition as an ESU (Turbek et al. 2023). Overall, our results show that Oosterschelde lobsters represent an isolated and largely self-recruiting population, for which effective localized conservation management is likely to be critical to the preservation of the stock and any fishery it supports (Dorant et al. 2022, Ellis et al. 2023, Turbek et al. 2023).

From our dataset, untangling potential genetic signatures of a reported historical transplantation to the basin, and any subsequent bottlenecks experienced within it, is not straightforward. In runs of snapclust using all loci, Oosterschelde individuals did not share ancestral clusters with individuals from any other site; they formed a single, unique cluster up to a value of $K = 20$ (after which individuals were partitioned into two or more separate clusters, though still discrete from other sites). At this level of clustering, all other sampling areas were split between groups, with individuals assigned to up to 11 different clusters (mean = 6.33) (Supplementary Material S6.3). It may be noteworthy that the site with the next tightest clustering integrity was Helgoland (all individuals to two clusters). Around the time of the reported 1962/3 bottlenecking event in Oosterschelde lobsters, Helgoland's lobster stock is thought to have collapsed as a consequence of extensive Allied bombing of the area during and after World War II (Schmalenbach et al. 2009). Our Helgoland sample also showed slightly below-average allelic richness and polymorphisms, so relative uniformity in cluster assignments even at inflated values of K could indicate narrow ancestry as a result of a recent population bottleneck, though this does not help to attribute spatial origins of ancestry.

Our other results were similarly inconclusive in terms of supporting the origin theories of the Oosterschelde lobster stock as claimed in historic literature. Using PCAs incorporating both neutral and outlier loci, Oosterschelde individuals grouped slightly nearer to Scandinavian samples than to other Atlantic samples on the PC-1 axis, which explained the highest proportion of variation (Fig. 3a and b). However, this pattern was only very marginal with neutral loci, the most suitable markers with which to assess ancestry. With outlier SNPs, there was even some overlap among spatial clusters between individuals of Oosterschelde and Scandinavian origin. At many outlier loci, our Oosterschelde sample produced allele frequencies that were consistent with other nearby sites, or at least its location somewhere between Atlantic and Scandinavian sites. However, at four outlier loci whose spatial pattern seemed foremost to differentiate Scandinavian samples from the rest, Oosterschelde had allele frequencies that were much more consistent with Scandinavian samples than other North Sea sites (Supplementary Material S4). It is possible that this could be a remnant of Scandinavian ancestry, although loci potentially under selection are not typically an effective indicator of shared descent; it is more likely that these similarities reflect common selection pressures in areas close to the limits of environmental tolerance in the species. Our evidence of a small loss of genetic diversity appears compatible with founder scenarios of Scandinavian lobsters establishing in the basin following their introduction as live-traded lobsters lost from a stricken trade vessel and/or flooded shore-side holding ponds (Havinga 1921, van Ysseldijk 1973). Overall, however, given the extent of their contemporary divergence, our dataset is unsuited to any robust determination of whether or not Oosterschelde lobsters are descended from introduced animals of Scandinavian origin.

Signatures of range-wide and local adaptation

Alignment of outlier loci to the European lobster genome provided evidence of potential local adaptation in *H. gammarus*, including variants that might be important for adaptation in Oosterschelde lobsters. Of the 31 outlier loci identified as selection candidates, six aligned to exon regions of genes. Among gene regions aligning with outliers, locus *36 865_90* contained a missense variant (a point mutation that alters the transcribed codon to produce a different amino acid) aligning to the cation transporting ATP13A3 gene, regulation of which has been proposed to confer tolerance to environmental heat stress in northern pike (*Esox lucis*) (Jiang et al. 2022) and to cadmium toxicity in the red swamp crayfish (*Procambarus clarkii*) (Zhang et al. 2019). Similarly impactful was locus *23 186_32*, which contained both a missense mutation and a splice gene variant (which alters the number of nucleotides in an RNA splice site) to the 3′ UTR of KLF18. Differential expression of KLF genes has been attributed to antimicrobial functionality in the giant river prawn, *Macrobrachium rosenbergii* (Huang and Ren 2019). Another locus aligned to the 5′ UTR of the cyclophilin gene PPIL3, which is strongly expressed in haemocyte tissues and rapidly upregulated in response to pathogen exposure in the Suminoe oyster, *Crassostrea ariakensis* (Xu et al. 2016). Together, the gene annotations of these loci to KLF18 and PPIL3 suggest an importance in localized resistance to pathogens. Another locus aligned to the receptor tyrosine kinase DDR2, which has been linked to ovarian development in *M. rosenbergii* (Yang et al. 2023) and to wound repair and regeneration in the nematode *Caenorhabditis elegans* (Hisamoto et al. 2016). Overall, these data suggest that coding gene variation may underlie adaptive responses in lobsters to a range of biological processes, including thermal stress response, toxicity tolerance, and innate immunity. This comes with the caveat that reduced-representation sequencing only allows for the surveying of a small proportion of the genome. However, SNP variation has also been implicated in adaptive roles in similar functional pathways for the American lobster, *H. americanus* (Benestan et al. 2016, Dorant et al. 2022). Nonetheless, patterns of spatial variation in allele frequencies at these outlier loci fit the latitudinal (NE-SW) Atlantic cline and/or Scandi-

navian divergence rather than Oosterschelde divergence, so these are candidates for range-wide local adaptation rather than isolated adaptation specific to Oosterschelde lobsters (Supplementary Material S4).

In contrast, the outlier locus *18 037_40* was only variant in Oosterschelde lobsters, with the private allele carried by five of the seven individuals genotyped (4x heterozygotes; 1x homozygote). It aligned to a Tp53-induced glycolysis and apoptotic regulator (TIGAR)-like gene of the p53 tumour suppressor pathway, where a missense variant was identified. The TIGAR protein hydrolyses fructose-2,6-bisphosphate, the breakdown of which suppresses glycolysis (Li and Jogl 2009), and TIGAR-mediated inhibition of glycolytic rate can promote apoptosis, but can also promote a survival response in cells under hypoxia by maintaining glycolytic flux in the wake of oxidative stress (Cheung et al. 2012). In the Pacific white shrimp (*Litopenaeus vannamei*), TIGAR is mainly expressed in gill tissues when dissolved oxygen is plentiful, but is strongly upregulated in both hepatopancreas and gill tissues following initial exposure to hypoxic conditions, suggesting a role in cellular response to damage caused by environmental oxygen depletion (Camacho-Jiménez et al. 2019). Although lobsters have physiological coping mechanisms to withstand acute short-term hypoxia (i.e. during ecdysis; Clemens et al. 1999), the effects of sporadic and/or prolonged exposure to hypoxia are less well understood. In the Oosterschelde, a 2001 v mass mortality of 10 million kg of mussels (*Mytilus edulis*) was attributed to acute hypoxia resulting from a harmful algal bloom of *Phaeocystis globose* (Peperzak and Poelman 2008), while transplanted Oosterschelde lobsters persist in the near-stagnant Veerse Meer and Grevelingen basins, despite bottom waters in the latter reaching partial or complete anoxia during midsummer stratification (van Stralen and Smeur 2008, Seitaj et al. 2017, van Katwijk et al. 2023). The lobsters we collected from the Oosterschelde were captured near the storm-surge barrier in the estuary mouth (Fig. 1c), where North Sea water exchange is highest and hypoxia has not been observed (Capelle et al. 2021), but it is likely that mutations accumulated in other areas of the basin, or at other times in the past, would become widespread if conveying a fitness advantage (Lowe and Allendorf 2010). Overall, this link between an Oosterschelde private allele, whose variant is locally common and predicted to cause a change in transcription, and a gene involved in hypoxia-induced apoptotic signalling and oxidative stress response in decapods is suggestive of a genomic adaptation to local environmental conditions.

Conclusions

Our results show that Oosterschelde lobsters are extensively differentiated from surrounding stocks via both neutral and outlier loci, indicative of strong biophysical and demographic isolation (Lowe and Allendorf 2010). We detected signatures of both lost genetic diversity and novel adaptations to the conditions encountered in the Oosterschelde environment. With respect to fisheries, our findings indicate that it is advisable to monitor and manage the Oosterschelde lobster population as a discrete unit, on the assumption that the stock is entirely self-recruiting. Future studies should investigatewhether or not the current fisheries management regime adequately considers the unique characteristics of the population. Wherever possible, biological parameters of key importance to management should be directly estimated for Oosterschelde lobsters via local monitoring, rather than inferring parameters measured in other stocks (Ellis et al. 2023). To conserve putative local adaptations of the stock, any prospective hatchery restocking or other population enhancement scheme should be based solely on lobsters of native provenance, ideally as confirmed by genetic screening (Ellis et al. 2015, Jenkins et al. 2020). Further research using more powerful genomic methodologies on a larger sample of individuals, including temporal samples that account for the long generation time, could enable more powerful assessments of the interrogation and stability of adaptive variation in Oosterschelde lobsters to assess whether the population warrants designation as an adaptive conservation unit (Turbek et al. 2023). Such a dataset could also facilitate modelling approaches (i.e. Gutenkunst et al. 2009, Excoffier et al. 2013) and more reliable estimates of effective size (i.e. Santiago et al. 2020, 2024) that may shed more light on their demographic history and current conservation status.

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Author contributions

C.D.E., T.L.J., and J.R.S. conceived the study; C.D.E., T.L.J., and M.R.v.S. conducted and coordinated sample collection; C.D.E. and T.L.J. conducted laboratory work; C.D.E., J.R.P., and T.L.J. developed and undertook the analysis; C.D.E. and J.R.P. wrote the original manuscript draft; and C.D.E., J.R.P., T.L.J., M.R.v.S., N.A.S., J.S., and J.R.S. edited, revised and refined the manuscript.

Supplementary data

Supplementary material is available at *ICES Journal of Marine Science* online.

Conflict of interest: All authors declare no conflicts of interest.

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Data availability

Raw DNA sequence data are available from the NCBI Sequence Read Archive (SRA) database (BioProject Accession IDs: PRJNA954007, https://www.ncbi.nlm.nih.gov/bioproject/PRJNA954007). SNP genotypes in VCF format and R code

used to analyse data are available from GitHub (https://github.com/TheLobsterDr/OosterscheldeLobsterDivergence).

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