


Current genetic status, temporal stability and structure of the remnant wild European flat oyster populations: conservation and restoring implications

Manuel Vera^{1,2}  · Jens Carlsson^{3,4} · Jeanette EL Carlsson^{3,4} · Tom Cross⁴ · Sharon Lynch⁴ · Pauline Kamermans⁵ · Antonio Villalba^{6,7} · Sarah Culloty⁴ · Paulino Martinez¹

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Abstract The flat oyster (*Ostrea edulis*) is one of the most appreciated molluscs in Europe, but natural beds have been greatly reduced due to harvesting and the effects of the parasite *Bonamia ostreae*. Characterization of current wild populations is required to develop long-term bed restoration programmes by enhancing genetic diversity and tolerance to bonamiosis. Oysters from different locations corresponding to the main natural beds from Denmark, The Netherlands, England, Ireland, France and Spain, including two different cohorts per location were sampled in 2011 and 2013. Sixteen microsatellite loci were used to study temporal and geographical genetic structure. Temporal variation was low, although sometimes significant probably due to high variance in reproductive success among individuals.

Conversely, samples from different countries showed much higher genetic divergence, and Ireland and France presented differences among locations within country. Clustering analyses grouped samples into three main geographical regions, associated with oceanic fronts: one group constituted by The Netherlands and Denmark; another by France, Ireland and England; and a third one exclusively by Spain. Effective population sizes (N_e) within regions were high (>1000), which reflects population stability and low levels of genetic drift. The presence of regional genetic structure shows the potential for local adaptation of *O. edulis* and suggests caution when transplanting individuals, especially between distant geographical regions.

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M. Vera and J. Carlsson have contributed equally to this work and are first co-authors.

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✉ Manuel Vera
manuel.vera@usc.es

¹ Departamento de Genética, Facultad de Veterinaria, Universidad de Santiago de Compostela, Campus de Lugo, 27002 Lugo, Spain

² Laboratori d'Ictiologia Genètica, Departament de Biologia, Facultat de Ciències, Universitat de Girona, Campus Montilivi, 17071 Girona, Spain

³ Area 52 Research Group, School of Biology and Environmental Science, University College Dublin, Dublin, Ireland

Introduction

The European flat oyster (*Ostrea edulis*) is a sessile, protandrous hermaphroditic, filter-feeding bivalve mollusc with a wild distribution ranging from Norway to Morocco in the Atlantic Ocean, and across all the Mediterranean Sea up to the Black Sea (Jaziri 1990; Lallias et al. 2010a). *O. edulis* is a highly appreciated aquaculture species in Europe

⁴ School of Biological, Earth and Environmental Sciences/ Aquaculture and Fisheries Development Centre, University College Cork, North Mall, Cork, Ireland

⁵ Institute of Marine Research and Ecosystem Studies, Wageningen UR, PO Box 77, 4400 AB Yerseke, The Netherlands

⁶ Centro de Investigacións Mariñas, Consellería de Pesca e Asuntos Marítimos, Xunta de Galicia, 36620 Vilanova de Arousa, Spain

⁷ Departamento de Ciencias de la Vida, Universidad de Alcalá, 28871 Alcalá de Henares, Spain

and, due to its economic potential, has been introduced in other parts of the world such as USA, Canada, Japan and South Africa (Launey et al. 2002; FAO 2015). This mollusc species has had a historical importance as part of the human diet for centuries, being reared since ancient civilizations such as Greek and Roman (Gonzalez-Serrano 2001). However, during the first half of the twentieth century overharvest and habitat destruction have caused severe decline of flat oyster populations throughout its native distribution (Laing et al. 2006). Further, two diseases (caused by the Protozoa *Marteilia refringens* and *Bonamia ostreae*), spread in the early 1970s and 1980s, drastically reduced the production of *O. edulis* in the majority of European traditional rearing areas (Lallias et al. 2007). Despite improved management practices and intensive enhancement programmes, the production of *O. edulis* has remained low since then. Thus, oyster production declined from the 10,000–30,000 tons/year in the 1960s to c. 2000 in 2012 (FAO 2015). Selective breeding programmes, mainly for resistance to *B. ostreae*, were successfully developed in different European countries (Naciri-Graven et al. 1998; Culloty et al. 2004; da Silva et al. 2005) which indicates a substantial genetic variance underlying this trait. Thus, restoring *O. edulis* wild populations or improving production using pathogen-resistant seed could be feasible.

Genetic characterization of natural and cultured beds is required to develop long-term bed restoration and to identify the raw material for enhancing tolerance to bonamiosis in breeding programmes. Moreover, native oyster reefs provide many ecosystem services including water filtration, food and habitat for many animals, shoreline stabilization, coastal defence and fisheries (Beck et al. 2011), being essential for habitat management and conservation. The OSPAR (from Oslo and Paris conventions) Commission, which is in charge of the protection and conservation of the North-East Atlantic resources, has listed *O. edulis* as one of the threatened species in the region. This commission has also provided management recommendations for wild oyster beds inside Marine Protected Areas (OSPAR 2008). Pilot experiments using oyster spat transplantation to re-establish degraded areas have been recently performed and demonstrated higher settlement success in non-harrowed areas (Bromley et al. 2016).

Over the last three decades, genetic diversity and geographical structure of flat oyster wild populations have been analysed using different molecular markers such as allozymes (Blanc et al. 1986; Jaziri 1990; Saavedra et al. 1993, 1995), mitochondrial DNA (mtDNA) (Diaz-Almela et al. 2004) and microsatellites (Launey et al. 2002; Sobolewska and Beaumont 2005; Beaumont et al. 2006; Lallias et al. 2010a). Based on allozyme data from a limited number of sample locations, Jaziri (1990) suggested that, after the last glacial Pleistocene episode, Mediterranean

oysters colonized the Atlantic areas. Saavedra et al. (1993, 1995) analysed a larger number of locations (19) encompassing the entire natural range of the species (excluding the Black Sea) and suggested the presence of two population units allopatrically differentiated and associated with each basin (i.e. Atlantic and Mediterranean), which would have merged in the Strait of Gibraltar since the last glaciation. Further studies across the distribution range including the Black Sea did not detect such transition between areas using either microsatellite loci (Launey et al. 2002) or mtDNA (Diaz-Almela et al. 2004). High genetic diversity has been reported in oyster populations, being generally lower in the Atlantic than in the Mediterranean area. This has been associated with shorter favourable periods for reproduction and stronger effects of parasites in the Atlantic region (Launey et al. 2002), which would have caused a reduction of the effective population size (N_e). Data also suggest that the genetic structure of flat oyster follows an isolation by distance model (Saavedra et al. 1995; Launey et al. 2002; Diaz-Almela et al. 2004). In summary, available information suggests that *O. edulis* does not constitute a single panmictic unit throughout its natural range despite the high gene flow mediated by pelagic larvae (8–10 days pelagic larval duration; Launey et al. 2002). In the Atlantic area, Sobolewska and Beaumont (2005) using four microsatellite loci found weak population differentiation among locations, and argued that the Atlantic region would constitute a single genetic resource relevant for conservation. However, Beaumont et al. (2006), using six loci, identified genetically isolated groups within the Atlantic region and suggested that they should be taken into account for management and that movements of live oysters within this region should be avoided. Genetic variability in wild and pond-produced locations, as well as in hatchery-produced stocks (four locations for each group, 12 locations in total), was estimated using five microsatellite loci (Lallias et al. 2010a). Hatchery stocks showed lower genetic diversity, high differentiation from wild locations and signs of inbreeding. Thus, Lallias et al. (2010a) argued that restocking of wild populations with hatchery stocks could be detrimental for their conservation. However, pond production systems could represent a valuable alternative source for restocking wild *O. edulis* populations because pond production seems to maintain genetic diversity better than other culture procedures (Lallias et al. 2010a).

The differences observed in allele frequencies and genetic diversity (i.e. number of alleles) among local stocks may reflect a certain isolation and determine variation in effective population size (N_e), a key parameter to evaluate population viability and conservation (Sobolewska and Beaumont 2005; Beaumont et al. 2006; Vercaemer et al. 2006). The effective number of breeders (N_b) within populations can be low as observed in two French flat

oyster populations ($N_b = 10\text{--}20$ individuals), supporting the hypothesis of sweepstake reproductive success in the species (Hedgecock et al. 2007). The lowest N_b estimates were observed in hatchery stocks; pond-produced oysters had intermediate estimates; and wild populations showed significantly higher N_b estimates (Lallias et al. 2010a). However, variance in reproductive success was observed in both hatchery and wild conditions (Lallias et al. 2010b). Moreover, sequencing of mitochondrial DNA showed a reduced number of effective females ($N_{e\text{♀}}$) within the species (Diaz-Almela et al. 2004) mainly due to the higher variance in female reproductive success (Boudry et al. 2002) and to the male biased sex ratio during reproductive season (3♂:1♀; Le Dantec and Marteil 1976). This bias could be caused by flat oyster being protandrous hermaphrodites and the higher incidence of *B. ostreae* on 2–3-year-old individuals, mostly females, further skewing the sex ratio (Culloty and Mulcahy 1996). Hence, differences in sex ratio between Bonamia-free and Bonamia-infected areas could be expected, an observation not documented to date; thus, further information is needed.

Despite the significant effort to elucidate genetic structure of flat oyster, no information is available on temporal genetic variance to date, essential to evaluate the reproductive bias whereby cohorts can substantially differ from the parental generation (Hedgecock et al. 2007). Moreover, the number of microsatellite loci used in previous studies was low (i.e. ≤ 6 microsatellite loci), which may have limited the potential to detect subtle population structure inside the Atlantic region. The aim of this study is to analyse the current genetic status of the main Atlantic remnant natural beds of the flat oyster (many of them infected by *B. ostreae*) with a larger number of microsatellite loci (16 markers) to describe both temporal and geographical structure related to oceanographic dynamics and connectivity. This information, including basic population parameters such as N_e , and $N_{e\text{m}}$ (i.e. number of effective migrants), is essential to assess the current status of flat oyster beds and to enable appropriate management and restoration (if necessary) of the wild resource which is also the raw material for breeding programmes.

Materials and methods

Biological material

Tissue samples were collected from a total of 1595 oysters across 16 wild natural beds throughout the European Atlantic Coast (Fig. 1) and stored in 100 % ethanol. Roughly 50 oysters were sampled twice from each location in 2011 and 2013, resulting in a total sample of about 100 individuals per location (Table 1). To ensure that two different cohorts

are included in the sampling design, adult individuals were collected in 2011 while spats were collected in 2013. All locations were affected by bonamiosis, except those in Denmark (DK-LHAVN and DK-BRED), where the parasite has not been reported (Flannery et al. 2014).

Molecular markers

Total DNA was extracted from gill tissue samples using Chelex-Proteinase K (Estoup et al. 1996), the eZN.A. E-96 mollusc DNA kit (Omega Bio-Tek) or standard phenol–chloroform procedures (Sambrook et al. 1989). A set of 13 anonymous microsatellite loci (Launey et al. 2002; Lallias et al. 2009) was selected based on their polymorphism and technical properties (e.g. low frequency of null alleles, high genotyping accuracy) using information from a pilot study (comprising 48 oysters, 24 from Ireland and 24 from Spain). Three additional recently developed microsatellite loci were included (OE-27, OE-03 and OE-11; Vera et al. 2015). The 16 loci were split into three PCR multiplexes and amplified using the Qiagen multiplex kit with labelled (FAM, NED, VIC and PET, Applied Biosystems) primers following manufacturer recommendations (detailed PCR multiplex conditions are shown in Table 2). The size of amplified fragments was analysed using GeneScan™ 500 LIZ® (Applied Biosystems) size standard on ABI 3730xl genetic analyzers (Applied Biosystems, Forest City, CA, USA) located in Queens University Belfast or in University of Santiago de Compostela (USC). Allele scoring was performed with GENEMARKER ver. 1.97 (Softgenetics, State College, PA, USA) at University College Cork (UCC) and with GENEMAPPER ver. 4.0 (Applied Biosystems, Forest City, CA, USA) at USC. Calibration of allele calling was performed between UCC and USC for all loci by genotyping in both platforms a reference panel of individuals composed of representative flat oysters across the sampled area in order to identify most of the allelic series per locus (reference individuals and genotypes for validation are available upon request to authors). Only individuals that showed successful amplification at ten or more loci were retained for further analyses.

Genetic data analyses

The software MICRO-CHECKER v. 2.2.3 (Van Oosterhout et al. 2004) was used to identify genotyping errors (stuttering, allele dropout and null alleles; 1000 randomizations). Allele frequencies, number of alleles, observed and expected heterozygosities, heterozygote excess and deficiency (exact test, 10,000 dememorizations and 5000 iterations) and tests for linkage disequilibrium (Fisher's method, 10,000 dememorizations and 5000 iterations) were estimated with the programme GENEPOP v. 4.0.9 (Rousset

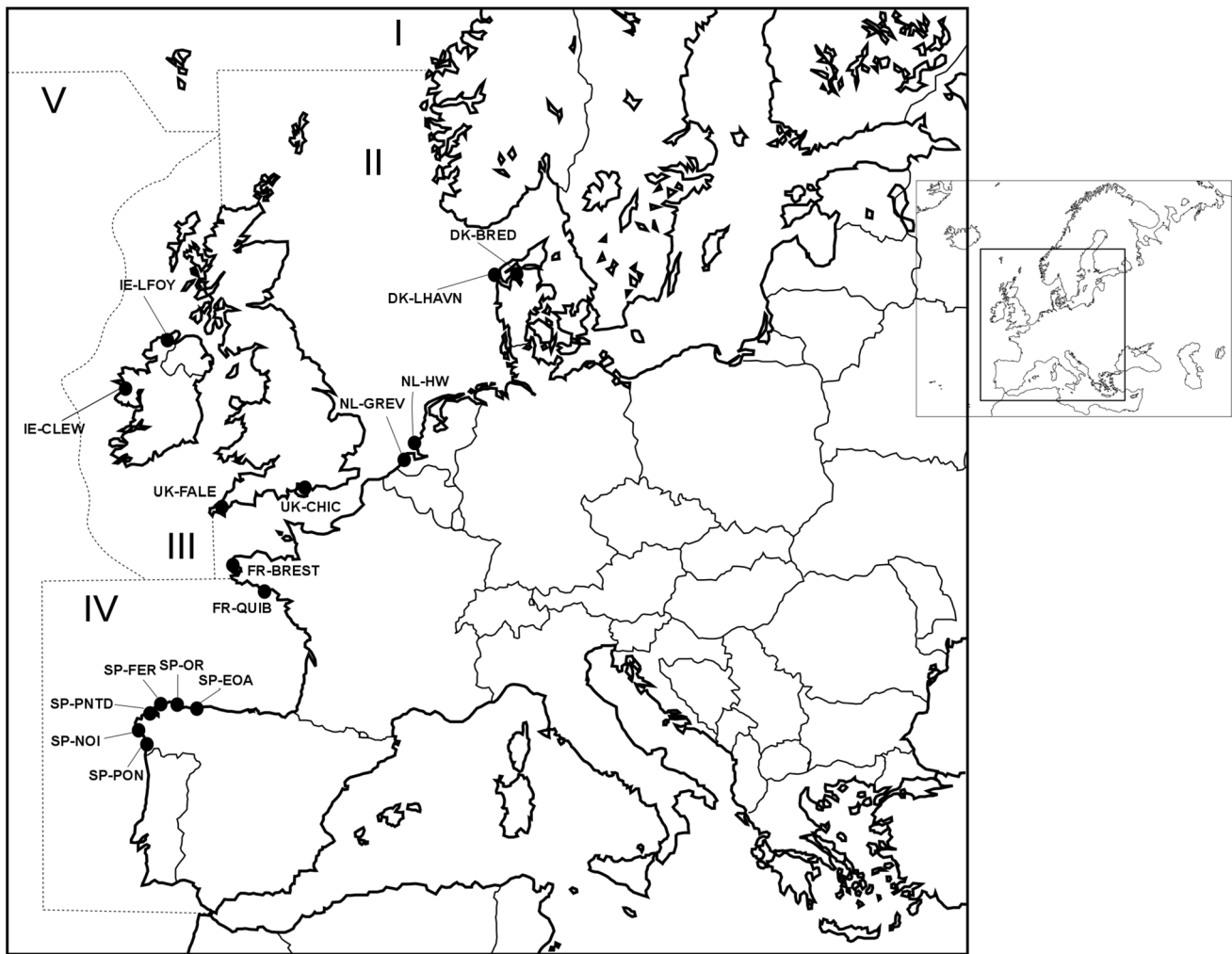


Fig. 1 Locations of *Ostrea edulis* analysed in the present study. Dotted lines and roman numbers show the OSPAR regions. Location codes are those shown in Table 1

2008). Allelic richness among locations was calculated using the FSTAT v. 2.9.3 programme (Goudet 2001). Weir and Cockerham (1984) unbiased estimator of Wright's (1951) F -statistics (F_{ST}) was used as implemented in the software package Microsatellite Analyser (MSA, Dieringer and Schlötterer 2003) to assess the relative component of genetic differentiation among locations. Partitioning of the observed structure into local/regional and temporal variation was performed using the AMOVA approach as implemented in the ARLEQUIN v. 3.5.1.3 software package (Excoffier et al. 2005). Their significance was tested with 10,000 permutations. Alpha values corrections for multiple tests were adjusted using Bonferroni correction. The STRUCTURE v. 2.3.3 software (Pritchard et al. 2000; Falush et al. 2007) was employed to sort individuals into clusters (using the admixture and correlated allele frequency model with 100,000 replicates and a burn-in length of 10,000) using K (number of clusters) from 1 to 32 (the

number of samples in the data) with five replicates for each K . The most likely K was assessed by plotting $\ln(PD)$ and implementing the ΔK method as in Evanno et al. (2005). Further, STRUCTURE analyses were performed applying the correction for null alleles in locations and loci for which MICRO-CHECKER indicated presence of null alleles (Falush et al. 2007) in an effort to avoid potential biases caused by null alleles. The CLUMPP v. 1.1.2 software (Jakobsson and Rosenberg 2007) was applied on STRUCTURE results to account for cluster label switching and to assign which clusters each run corresponded to (search options: greedy G' , using random input orders and 100 repeats). A neighbour-joining (NJ) tree was constructed from a matrix of Nei's D_a genetic distances between all pairs of populations (Nei 1987) using POPULATIONS 1.2.26 (Langella 2002) after pooling the weakly differentiated temporal replicates (see below). The robustness of the branches was tested with 1000 bootstrap replicates. The tree

Table 1 Sampling sites analysed in the present study, including geographical coordinates and the number of individuals (N) after quality filtering (individuals with >10 genotyped loci for each temporal replicate)

Country	Location	Code	Sampled years	N	Presence of <i>Bonamia</i>
Denmark	Lemvig Havn (56°33'05"N–08°18'09"E)	DK-LHAVN	2011	50	No
			2013	50	
	Nissum Bredning (56°36'55"N–08°24'00"E)	DK-BRED	2011	50	No
			2013	50	
The Netherlands	Grevelingen (51°44'70"N–03°58'14"E)	NL-GREV	2011	49	Yes
			2013	51	
	Hompelvoet (51°46'80"N–03°54'50"E)	NL-HW	2011	50	Yes
			2013	51	
England	Chichester Harbour (50°47'14"N–00°56'14"W)	UK-CHIC	2011	50	Yes
			2013	29	
	Fal Estuary (50°13'03"N–05°01'38"W)	UK-FAL	2011	50	Yes
Ireland	Loch Foyle (55°07'50"N–07°05'17"W)	IE-LFOYL	2011	50	Yes
			2013	40	
	Clew Bay (53°48'07"N–09°36'41"W)	IE-CLEWB	2011	50	Yes
			2013	50	
France	Brest (48°22'04"N–04°30'50"W)	FR-BREST	2011	49	Yes
			2013	50	
	Quiberon (47°28'40"N–03°07'13"W)	FR-QUIB	2011	51	Yes
			2013	53	
Spain	Eo Ria (43°31'22"N–07°02'20"W)	SP-EOA	2011	27	Yes
			2013	50	
	Ortigueira (43°42'36"N–07°52'18"W)	SP-OR	2011	47	Yes
			2013	50	
	Ferrol (43°27'36"N–08°12'24"W)	SP-FER	2011	44	Yes
			2013	50	
	Pontedeume (43°24'35"N–08°10'12"W)	SP-PNTD	2011	55	Yes
			2013	50	
Noia (42°46'48"N–08°56'07"W)	SP-NOI	2011	50	Yes	
		2013	47		
Pontevedra (42°25'02"N–08°42'24"W)	SP-PON	2011	50	Yes	
		2013	49		

was visualized using TreeView version 3.2 (Page 1996). In order to detect isolation by distance (IBD) pattern, correlation among geographical (measured as the shortest coastline between two locations in Km) and genetic (measured as $F_{ST}/1-F_{ST}$; Rousset 1997) distances was evaluated with a Mantel test with 10,000 permutations using NTSYS v.2.1 (Rohlf 1993). The programme MIGRATE-N v. 3.1.6 (Beerli and Felsenstein 2001) was used to estimate theta (Θ), which is equal to $4N_e\mu$ where N_e is the long-term (inbreeding) effective population size and μ is the mutation rate. From inspecting how the effective population size is estimated ($N_e = \Theta/4\mu$), it is clear that the mutation rate has a large effect on estimates of N_e . Mutation rates (μ) for microsatellites have been reported to range from 10^{-5} to 10^{-2} per locus and generation (Weber and Wong 1993). Hence, any estimate of N_e is subjected to large variation

depending on the specific mutation rate. However, mutation rates should be similar for each marker independently of the studied population. Therefore, no attempt was made to estimate actual effective population sizes and Θ estimates were instead used as proxies for measures of relative effective population sizes. In addition, MIGRATE-N was also used to estimate M , where $M = m/\mu$, m is the migration rate per generation and μ is the mutation rate. The number of immigrants per generation, $4N_e m$ (for nuclear data), can be estimated by multiplying Θ by M . In addition to the long-term effective population size, genotype data were analysed using the programme LDNe (Waples and Do 2008) employing the default setting to estimate contemporary N_e under random mating conditions. Genotype data from each individual sample were analysed with BOTTLENECK v. 1.2.02 [using the two-phase mutational model (TPM) with

Table 2 Multiplex reactions used in this study

Locus	Label	Multiplex	Forward primer	Reverse primer	References	PCR cycles	Primer concentration (μM)	Annealing temperature (°C)
Oed240	NED	1	GACTTACATAA-GCAAACCTCTT	ACTGGGCG-GTCACCAC-CTTGGGCC	Lallias et al. (2009)	30	0.15	59
OE27	FAM	1	CACAGTAA-GAACCG-GCTTGA	CCGTGTTTGCC-CAACTATCT	Vera et al. (2015)		0.20	
OE03	FAM	1	TTGATCCTCAAT-GATACTCGTG	GATGAGCGCGAT-GCTCTAAC	Vera et al. (2015)		0.20	
Oed325	PET	1	GAGACCTT-GATTC-GAAACTTCTTT	GTTTCTTCAC-GACATATCTAG-CACTTTTCA	Lallias et al. (2009)		0.20	
Oed202b	NED	1	GCGGGTAT-TACATTAG-CAATCC	GTTTCTTGTA-CATGGAAGTAG-GACAGTCA	Lallias et al. (2009)		0.20	
Oed202a	VIC	1	AAATTCAAAT-CACCGGAGGA	GTTTCTTCCTCC-CTGAATATCT-GTCCA	Lallias et al. (2009)		0.20	
OeduT5	VIC	2	CTTCGTTCTTG-TACGTAAGCG	TAGTGAATG-GTCTTGCAT TCC	Launey et al. (2002)	30	0.15	55
OeduO9	FAM	2	ATTCAAAT-GATTTTAG-GTTGG	ACTTCAATGTCT-GTTCTAATGG	Launey et al. (2002)		0.15	
OeduU2	NED	2	ACCAATGAACA-CAGATCACC	GAAAGAAATG-GAGGCAATAAC	Launey et al. (2002)		0.15	
Oed327	PET	2	CCGTTAGCCC-CATCAGATAA	TGGGGTGTAAG-TAATCTCCAG	Lallias et al. (2009)		0.15	
OeduJ12	PET	2	GCTGTATTTT-CATCAATTC-GAG	TCGTCACCTCC-CTCTCAGAG	Launey et al. (2002)		0.15	
Oed177a	FAM	3	TGCAAGAT-TAAAA-GGCAGCA	TCTGCACCTAATA-GACTGTTCTGA	Lallias et al. (2009)	30	0.15	55
Oed181	NED	3	TGGTCAGCT-AAACTGTTCAA	CAAGGC-CTTTTCAATAAT-GTACTGT	Lallias et al. (2009)		0.15	
Oed212b	VIC	3	TTGAAATGC-CGATGTCTGTC	TGCCTCTTTG-TAAAGTCTTG-TATATT	Lallias et al. (2009)		0.15	
Oed243	PET	3	GCCGCGAGCTGT AAT CAT A	CGGCTGACCGCTA-TATTTGT	Lallias et al. (2009)		0.15	
OE11	PET	3	GTGAGGCAGGT-GATTCCATT	CCCTTGCTCAGGT-GCTATTC	Vera et al. (2015)		0.20	

Number of PCR cycles, final primer concentrations and annealing temperature of the multiplex reaction are shown

an IAM and SMM variance of 30 and 70 %, respectively] to assess whether any of the samples showed indications of having undergone genetic bottlenecks. Finally, the LOSITAN software (Beaumont and Nichols 1996; Antao et al. 2008) was used to detect evidence for selection at individual loci (using 50,000 simulations with the switches “Neutral” mean F_{st} and ‘Force mean F_{st} ’) under both the infinite alleles model (IAM) and stepwise mutation model (SMM).

Results

Genetic variability

After retaining individuals with at least ten genotyped loci, 1591 individuals were available out of 1595 for further analyses. Control for null alleles and technical artefacts using MICRO-CHECKER indicated the presence of null alleles at a total of 52 evaluations out of 512 (16 loci × 32

samples), representing twice more than the 5 % expected by chance. The spread of null alleles across loci was rather even and not particularly prominent for any locus (Supplementary Table 1), except for Oed325 (12 out of 32 samples affected), OeduJ12 (8), Oed202a (7) and Oedu-U2 (7). The locus Oed325 showed a geographical pattern of null allele occurrence with null alleles observed in all samples except the Danish and Dutch samples. However, while there was a lack of consistent null alleles across most loci and samples, and given the low expected effect of null alleles on F_{ST} estimations and assignment tests accuracy (Carlsson 2008), analyses were done both with all loci included and with loci showing high frequency of null alleles removed. MICRO-CHECKER indicated stutter only at six occasions across the 512 tests (1 %), well within the 5 % expected by chance, and not associated with a specific locus or particular location. Further, MICRO-CHECKER did not indicate any large allele dropout. A total of 183 loci combinations of the 3840 tests (5 %) showed significant linkage (or gametic phase disequilibrium), a proportion very close to the expected false-positive rate of 5 %, with no particular pair of loci being linked in multiple locations (≤ 6 out of the 32 tested). Based on the relative low temporal variation (see below), temporal samples were pooled for descriptive statistical analyses (Supplementary Table 2). Number of analysed individuals per locus ranged from 61 at locus OE-11 in DK-LHAVN to 149 in UK-FAL at loci OE-03, Oed202a, Oed177a, Oed243 and OE-11. The number of alleles (N_a) ranged from three at locus OE-03 in SP-PNTD to 39 at locus Oed202a in SP-OR (mean = 19.8 ± 8.4). Allelic richness (A_r) varied from 2.58 at locus OE-03 in SP-PNTD to 33.99 at locus Oed202b in NL-GREV (mean = 19.8 ± 8.4). Expected heterozygosity (H_e) ranged from 0.243 at locus OE-11 in DK-BRED to 0.966 at locus Oed202a in SP-NOI (mean = 0.839 ± 0.178). Mean A_r and H_e values per pooled location ranged from 16.50 in SP-FER to 19.30 in IE-LFOYL (mean = 18.0 ± 1.0) and from 0.819 in NL-GREV to 0.855 in FR-QUIV (mean = 0.838 ± 0.010), respectively, with similar diversity levels for all locations when analysing temporal replicates separately and pooled temporal replicates (Table 3).

A number of loci deviated significantly from Hardy–Weinberg expectations in some samples after sequential Bonferroni correction (initial $\alpha = 0.05/16$, $P = 0.0031$). However, of the 41 observed deviations, 25 were associated with three loci. Locus OeduJ12 deviated in six samples, OE-03 deviated in nine samples and Oed325 in ten samples. While deviations at OeduJ12 and Oed325 were associated with null alleles in eight and twelve samples, respectively, OE-03 showed no indications of null alleles. There was no apparent geographical pattern to deviations from Hardy–Weinberg expectations for any locus except Oed325 that showed heterozygosity deficiency in UK-FAL,

IE-CLEWB and all samples from Spain and France; while no indications of heterozygote deficiency could be detected in UK-CHIC, IE-LFOYL or samples from the Netherlands and Denmark (Table 3, Supplementary Tables 1 and 2). Three loci showed indications of potentially being under directional selection. OE-27 and Oed325 showed footprints of directional selection under both IAM and SMM models, while Oed240 only showed footprints of positive selection under the SMM model after corrections for multiple tests using the false discovery rate (FDR) model included in LOSITAN software.

Temporal and geographical variation

Pairwise multilocus F_{ST} between years within locations ranged from 0.0000 to 0.0090. Five of the 16 analysed locations (UK-CHIC, DK-LHAVN, NL-GREV, NL-HW and FR-QUIB; pairwise F_{ST} values >0.0033) showed significant temporal variation after correction for multiple test (sequential Bonferroni correction, initial $\alpha = 0.05/16$, $P = 0.0031$). However, AMOVA analysis (Table 4) indicated that the temporal variability within location was relatively low ($F_{SC} = 0.0016$, $P < 0.001$) compared with the geographical variability among locations ($F_{CT} = 0.0061$, $P < 0.001$). Consequently, temporal replicates were pooled for further analyses. Global population differentiation (F_{ST}) was 0.0079 ($P < 0.001$; $F_{ST} = 0.0089$, $P < 0.001$ when temporal replicates were separated). When loci affected by null alleles were removed (i.e. Oed 325, OeduJ12, Oed202a and Oedu-U2), global F_{ST} was slightly higher (0.0084, $P < 0.001$; $F_{ST} = 0.0094$, $P < 0.001$ when temporal replicates were separated), probably reflecting the effect of decreased heterozygosity on F_{ST} caused by null alleles. All in all, in accordance with previous observations (Carlsson 2008) loci with null alleles did not cause significant distortion of data and were subsequently retained for downstream analyses.

The closest geographical samples (i.e. from each country) showed no significant multilocus F_{ST} , while they showed significant values regarding those more distant after correction for multiple tests (sequential Bonferroni correction, initial $\alpha = 0.05/71$, $P = 0.0007$; Supplementary Table 3). No within-country differentiation could be observed with the exceptions of the two locations within Ireland ($F_{ST} = 0.0053$, $P < 0.001$) and within France ($F_{ST} = 0.0011$, $P < 0.001$).

In spite of the low within-country differentiation, AMOVA analyses (Table 4) indicated significant variation between locations within countries ($F_{SC} = 0.0012$, $P < 0.001$), but the variation among countries was nearly six times higher ($F_{CT} = 0.0068$, $P < 0.001$). The STRUCTURE analysis assessed K from 1 to 32 (the number of samples) and the resulting $\ln(\text{PD})$ plot gave very similar

Table 3 Genetic variability at 16 microsatellite loci in the studied locations for temporal replicates and pooling them

Location	Year sampled	<i>N</i>	<i>Na</i>	<i>Ar</i>	<i>Ho</i>	<i>He</i>	<i>F_{IS}</i>
DK-LHAVN	2011	50	15.4	11.5	0.819	0.842	0.0272
	2013	50	15.9	11.8	0.798	0.822	0.0288
	Pooled	100	18.7	16.8	0.795	0.820	0.0305
DK-BRED	2011	50	14.9	11.2	0.779	0.836	0.0680
	2013	50	16.8	12.1	0.816	0.838	0.0266
	Pooled	100	18.7	17.7	0.786	0.824	0.0461
NL-GREV	2011	49	16.1	11.7	0.798	0.812	0.0174
	2013	51	15.9	11.7	0.806	0.823	0.0206
	Pooled	100	18.5	18.2	0.802	0.819	0.0211
NL-HW	2011	50	16.3	12.2	0.777	0.824	0.0576
	2013	51	17.9	12.8	0.813	0.833	0.0243
	Pooled	101	20.8	18.0	0.794	0.830	0.0434
UK-CHIC	2011	50	16.4	12.1	0.809	0.829	0.0239
	2013	29	15.8	13.1	0.839	0.838	-0.0008
	Pooled	79	19.7	19.3	0.821	0.837	0.0191
UK-FAL	2011	50	17.0	12.7	0.798	0.847	0.0585
	2013	99	21.9	13.3	0.833	0.846	0.0159
	Pooled	149	23.8	18.7	0.821	0.848	0.0318
IE-LFOYL	2011	50	16.0	11.9	0.801	0.838	0.0441
	2013	40	16.0	12.5	0.806	0.850	0.0510
	Pooled	90	19.1	18.9	0.801	0.843	0.0498
IE-CLEWB	2011	50	16.1	12.3	0.827	0.836	0.0099
	2013	50	16.2	12.0	0.818	0.844	0.0311
	Pooled	100	18.9	16.6	0.819	0.837	0.0215
FR-BREST	2011	49	16.4	18.1	0.819	0.849	0.0357
	2013	50	12.5	13.1	0.812	0.854	0.0486
	Pooled	99	20.6	19.1	0.813	0.852	0.0457
FR-QUIB	2011	51	17.3	12.8	0.813	0.850	0.0433
	2013	53	18.5	13.6	0.833	0.855	0.0265
	Pooled	104	21.3	18.3	0.824	0.855	0.0363
SP-EOA	2011	27	14.8	12.7	0.761	0.829	0.0824
	2013	50	17.7	13.0	0.825	0.845	0.0235
	Pooled	77	19.6	19.1	0.800	0.841	0.0487
SP-OR	2011	47	16.8	17.4	0.812	0.847	0.0412
	2013	50	12.7	12.7	0.800	0.836	0.0426
	Pooled	97	19.8	17.3	0.804	0.842	0.0451
SP-FER	2011	44	16.1	12.6	0.808	0.843	0.0420
	2013	50	17.1	12.6	0.779	0.831	0.0621
	Pooled	94	18.9	16.5	0.792	0.837	0.0537
SP-PNTD	2011	55	17.3	12.6	0.812	0.828	0.0197
	2013	50	16.9	12.5	0.811	0.845	0.0404
	Pooled	105	19.4	18.3	0.811	0.837	0.0310
SP-NOI	2011	50	16.7	12.6	0.813	0.844	0.0367
	2013	47	17.6	13.0	0.801	0.849	0.0568
	Pooled	97	19.9	19.0	0.806	0.847	0.0484
SP-PON	2011	50	17.6	13.0	0.806	0.847	0.0494
	2013	49	17.1	13.0	0.826	0.848	0.0253
	Pooled	99	19.4	16.8	0.815	0.848	0.0389

Number of genotyped individuals (*N*), mean number of alleles detected (*Na*), mean allelic richness standardized for the smallest sample size (*Ar*), mean observed heterozygosity (*Ho*), mean unbiased expected heterozygosity (*He*, Nei 1978), inbreeding coefficient (*F_{IS}*) are shown

Table 4 AMOVAs results for the different hierarchical models performed

	<i>F</i> -statistic	Variance component	% Variation
I—Temporal			
Among locations (F_{CT})	0.00605***	0.03907	0.60
Between temporal replicates within location (F_{SC})	0.00163***	0.01050	0.16
Within samples		6.41341	99.23
II—Countries			
Among countries (F_{CT})	0.00677***	0.04378	0.68
Among locations within country (F_{SC})	0.00116***	0.00748	0.12
Within locations		6.41859	99.21
III—Regions			
Among regions (F_{CT})	0.00741***	0.04798	0.74
Among countries within region (F_{SC})	0.00167***	0.01076	0.17
Within countries		6.41859	99.09

Model I used unpooled samples, while models II and III pooled temporal replicates for the analyses (see text)

*** $P < 0.001$

support for K from 1 to 14 (Supplementary Fig. 1). However, when using the ΔK approach by Evanno et al. (2005), $K = 2$ showed the highest support (Supplementary Fig. 2). Scenarios from $K = 2$ to $K = 5$ were further analysed using CLUMPP (Fig. 2). At $K = 2$ it was clear that the Spanish and Danish/Dutch samples formed separated clusters with the English/Irish/French samples constituting an intermediate/mixed cluster. At $K = 3$, the three regions suggested above were evident as independent clusters (cluster I: Danish/Dutch, cluster II: English/Irish/French and cluster III: Spanish samples), while with $K = 4$ and $K = 5$, neither further geographical structure and additional substructuring nor temporal differentiation could be detected (Fig. 2). As expected, the same results were rendered when correction for null alleles was applied for STRUCTURE analyses (Supplementary Fig. 3). As no further geographical or temporal structure could be observed after $K = 3$, we performed an AMOVA analysis using the clusters from this scenario (Table 4), which indicated a significant amount of variation within these three regions ($F_{SC} = 0.0017$, $P < 0.001$), but with five times higher variation among them ($F_{CT} = 0.0074$, $P < 0.001$). In spite of the low bootstrap values shown for the robustness of the branches, the NJ tree also corroborated the presence of three different clusters geographically grouped (Fig. 3). Finally, a significant correlation among geographical and genetic distance matrices was detected suggesting isolation by distance ($r = 0.82439$, $P = 0.0001$; Supplementary Fig. 4).

Demographics and gene flow

The BOTTLENECK analysis did not show indications of genetic bottlenecks in any sample when assuming the TMP model of mutation (Wilcoxon test resulting in

nonsignificant probabilities after sequential Bonferroni correction for multiple tests initial $\alpha = 0.05/32$, $P = 0.0016$). Estimates of effective population size per location obtained with LDNe (allowing for a minimum allele frequency of 1 %) generated negative N_e for some samples which were interpreted as ∞ , and hence, the LDNe method could not successfully produce estimates for these samples. In samples where LDNe was able to estimate N_e the estimates ranged from c. 1000 to 17,000 (Supplementary Fig. 5). Most locations showed 95 % confidence intervals (CI) that included infinity, while the lower 95 % CIs ranged from 700 to 8400 (Supplementary Fig. 5). The regional subdivision with $K = 3$ suggested by STRUCTURE and NJ analyses was also analysed with LDNe. While LDNe could only generate a N_e point estimate for the Danish/Dutch group (22,800), the programme generated 95 % CIs for all groups that ranged from 2600 to ∞ (Table 5). Similarly, the long-term relative inbreeding effective population size was analysed for these three groups with MIGRATE-N resulting in Θ estimates ranging from 0.072 in the Danish/Dutch group to 0.098 in the English/Irish/French group (Fig. 4). The scale and directionality of global gene flow was also estimated with MIGRATE-N showing that the Danish/Dutch group received more migrants than those generated, while gene flow was symmetric between the English/Irish/French and Spanish groups (Fig. 5).

Discussion

Genetic diversity and temporal stability

Preservation of genetic diversity is vital to allow natural populations to adapt to environmental changes and

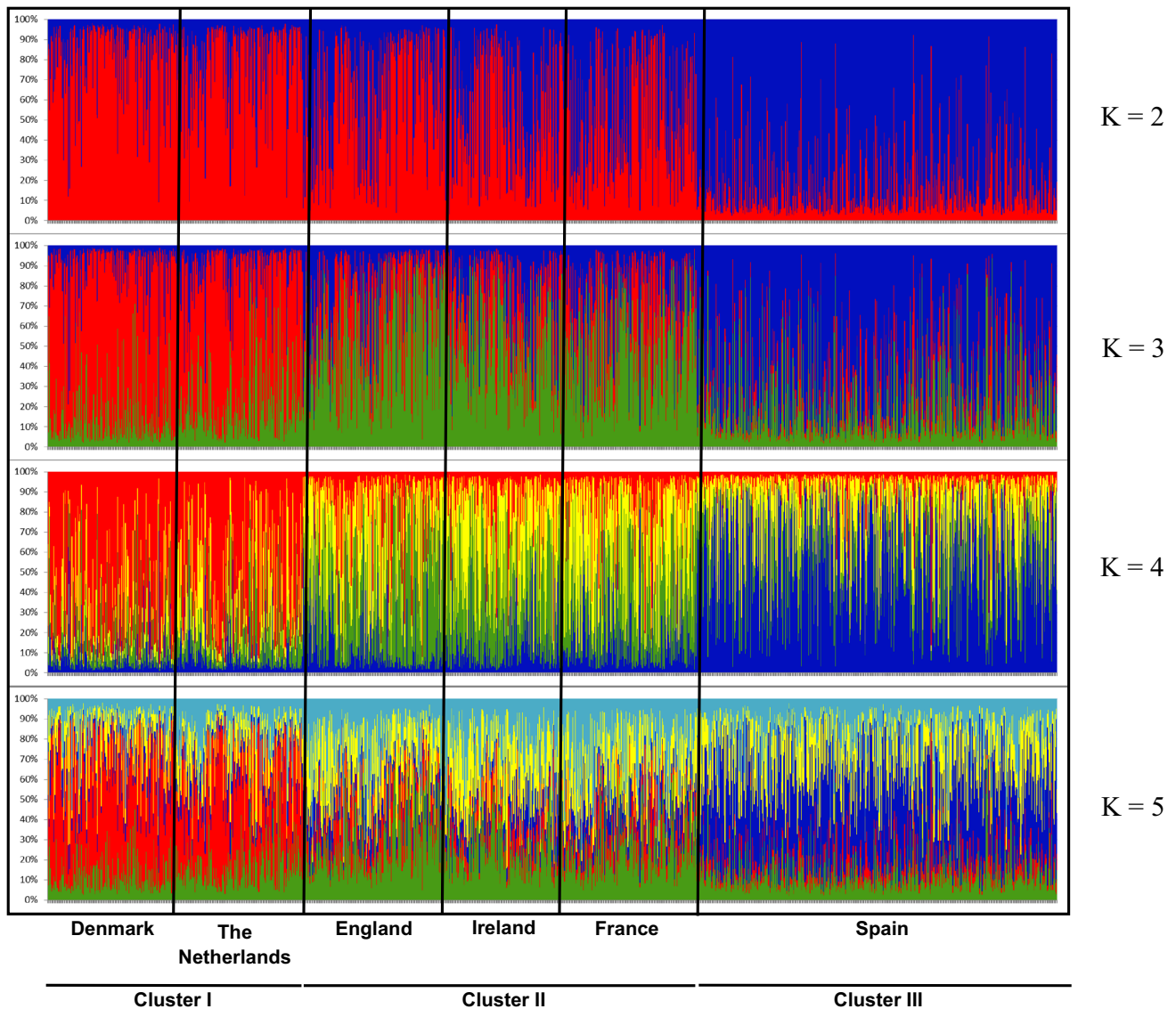


Fig. 2 Bayesian analyses of population structure performed with STRUCTURE. Each vertical bar represents one individual, and the colour proportion for each bar represents the posterior probability of

assignment of each individual to the different clusters (K) inferred by the programme

maintained population demographics rely on wild individuals to counterbalance depletion caused by overexploitation or diseases (Frankham et al. 2002). Moreover, maintaining natural genetic variability is also important for farmed species, like *O. edulis*, as this variability constitutes the raw material for broodstock trait variability and selective breeding (Lallias et al. 2010a). The current study reports similar genetic diversity within all analysed flat oyster populations ($Ar > 16$, $He > 0.800$) in accordance with previous reports from the Atlantic region (Sobolewska and Beaumont 2005; Beaumont et al. 2006; Lallias et al. 2010a).

The frequency of null alleles (i.e. non-amplified alleles by PCR due to mutations in primer annealing sites) across

most loci was low except for Oed325 and OeduJ12, and their impact on population structure was negligible. This presence of null alleles in microsatellite loci has previously been reported for flat oyster (Beaumont et al. 2006) and in general for molluscs (McGoldrick et al. 2000; Hedgecock et al. 2004; Diz and Presa 2008; Pino-Querido et al. 2015). Single nucleotide polymorphisms (SNPs) are widespread in mollusc genomes, which increases the probability of null alleles because of primer annealing failure in adjacent regions. While SNP frequency in *O. edulis* range from one SNP every 47 bp in noncoding regions to one every 76 bp in coding regions (Harrang et al. 2013), it can be even higher for other oyster species. For example,

Fig. 3 Neighbor-Joining population tree based on Nei's standard genetic distance (D_a). The numbers of the branches indicate the number of times (percentage) a clade on the original tree is present in the trees estimated from 1000 replicates. Suggested clusters by STRUCTURE analyses are included

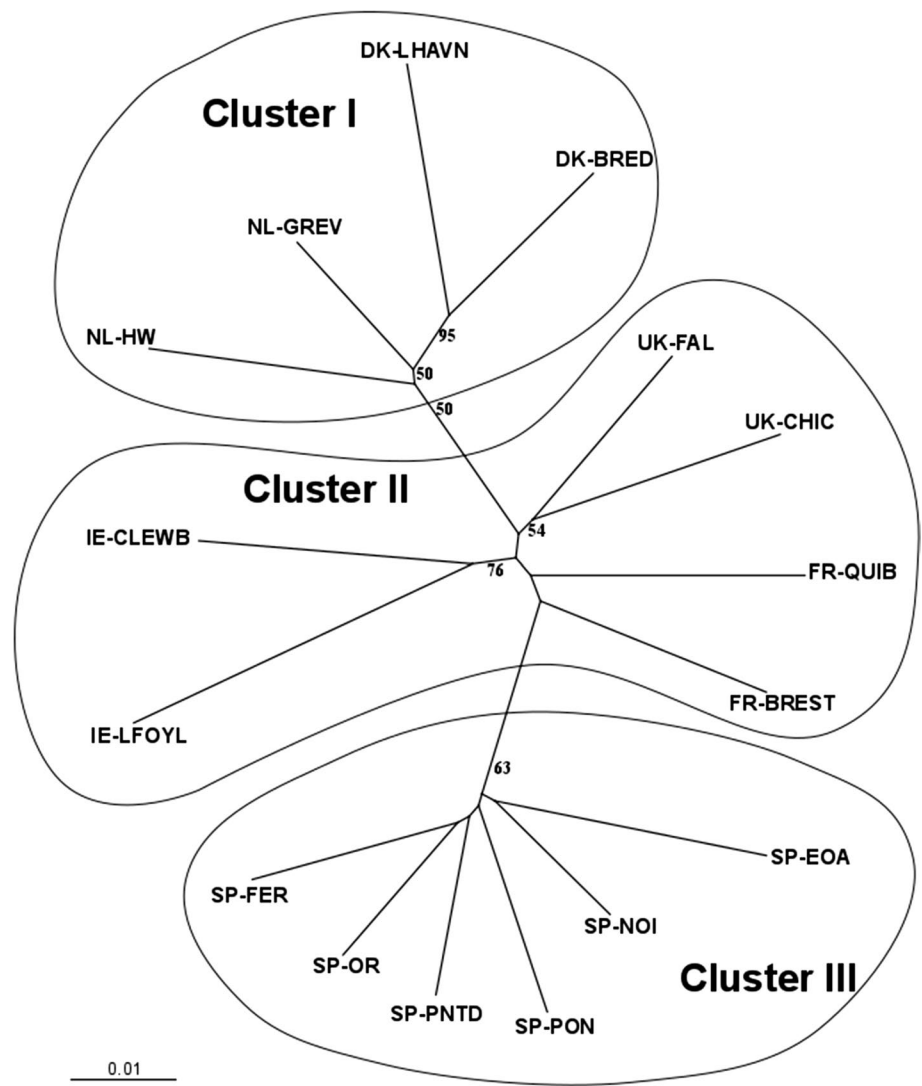


Table 5 Estimations of effective population sizes (N_e) and 95 % confidence intervals (CI) within each suggested STRUCTURE region using the programme LDNe

Region	N_e	95 % CI low	95 % C.I. high
DK/NL	22,792.5	2640.6	∞
UK/IE/FR	n/a	9325.3	∞
SP	n/a	5966.4	∞

one SNP every 60 bp in coding regions and each 40 bp in noncoding regions have been reported for *Crassostrea gigas* (Sauvage et al. 2007), and even higher in *C. virginica* (1 SNP every 20 bp; Zhang and Guo 2010). Despite the high frequency of SNP that can lead to inflated deviations from HW expectations, the effects of null alleles on F_{ST} estimations and assignment tests are low and in line with data presented in Carlsson (2008). The current study corroborates this observation, and the inclusion of loci

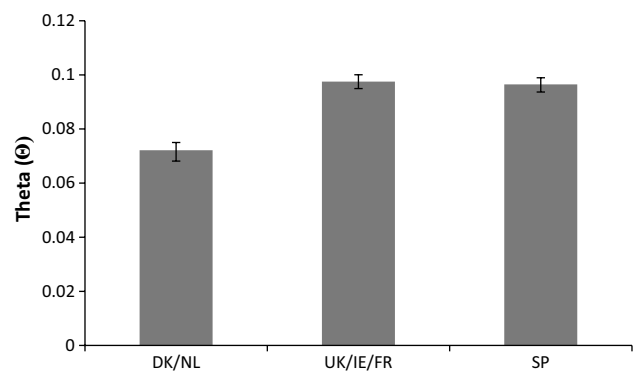


Fig. 4 Estimation of theta value (θ) representing relative effective population sizes (N_e) for the three detected clusters

with moderate null allele frequencies can increase accuracy of estimated population parameters by diminishing inter-locus variance.

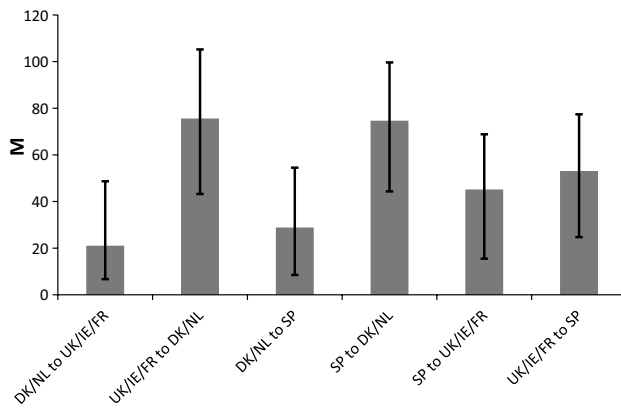


Fig. 5 Estimation of median migration (M) values among detected clusters. The bars show the 95 % confidence interval (CI) for each value

Effective population size (N_e) is a key parameter for assessing the viability of populations for conservation (Frankham et al. 2002; Sobolewska and Beaumont 2005). The reported genetic differences among some temporal replicates in flat oyster (i.e. cohorts) may suggest variance in reproductive success among cohorts, thus reducing N_e (Lallias et al. 2010b). Flat oyster has shown sex-biased ratio (3♂:1♀) at reproductive season (Le Dantec and Marteil 1976), although proportion of females in our samples varied between 20 and 100 % depending on local environmental factors which play an important role on sex (e.g. Orton 1927; Cole 1942; Korrington 1957). Controlled experiments of temperature and photoperiod showed that oysters are predominantly females at the beginning of spawning season when the water temperature is colder, while the frequency of males increase and are more frequent when the temperatures are warmer at the end of the spawning season (Joyce et al. 2013). Skewed sex ratio on populations can lead to decreasing N_e (Wright 1931). Hedgecock et al. (2007) hypothesized sweepstakes on reproductive success (i.e. low number of progenitors succeeds in replacing an entire population, while the majority fails to procreate) with very low effective number of breeders (N_b) estimates (10–20), hence increasing inbreeding. Moreover, N_b has been shown to be reduced in hatchery stocks when compared to wild populations (Lallias et al. 2010a). Most of our temporal cohorts showed genetic temporal stability, which suggests that unequal spawning events are less frequent than previously suggested (Hedgecock et al. 2007; Lallias et al. 2010b). Additionally, although *B. ostreae* has drastically reduced *O. edulis* production during the twentieth century (Lallias et al. 2007), no signs of significant bottleneck have been detected. Estimates of N_e per location were around or higher than 1000 in the most conservative estimates within regions, with N_e values higher than 2500 by region.

In conclusion, these results show low impact of genetic drift on flat oyster populations and suggest the viability of populations, since most adaptive potential has likely been retained (Frankham et al. 2002). Similarly, genetic studies of marine species suffering a mass mortality by pathogens (e.g. sea urchin, *Diadema antillarum*) or overfishing (e.g. European spiny lobster, *Palinurus elephas*) were unable to detect bottleneck signals, and despite the severe reductions in census population size, the species retained large and stable historical N_e and high diversity levels (Lessios et al. 2001; Palero et al. 2011).

Population structure and connectivity at macro- and microgeographical scale

Despite the low level of temporal variation observed among flat oyster cohort, spatial differentiation was close to four times higher than temporal variation (F_{ST} 0.0061 vs. 0.0016). Three consistent differentiated clusters (Danish/Dutch cluster, English/Irish/French cluster and Spanish cluster) were suggested by STRUCTURE analyses and supported by NJ tree and AMOVA. Isolation by distance (IBD) pattern has been suggested for *O. edulis* in its whole natural distribution range (e.g. Launey et al. 2002), with slight, but significant structure inside the Atlantic area (Beaumont et al. 2006). Our study confirmed structuring in the Atlantic region that follows isolation by distance (IBD)—a pattern not previously reported (Sobolewska and Beaumont 2005; Beaumont et al. 2006). The reason why the current study was able to detect IBD in the Atlantic may be related to the higher power associated with a high number of molecular markers and large number of sampling locations considered in our study. Beaumont et al. (2006) grouped locations from French Brittany and The Netherlands using Nei's (1978) unbiased genetic distance (D) and reported a pairwise F_{ST} between Norway and The Netherlands (0.051) that was lower than that observed between The Netherlands and France (0.056). The discrepancy with our data may be related to sampling effort between both studies. Beaumont et al. (2006) analysed northern Scottish populations in British Isles and three continental sites corresponding to Norway, The Netherlands and France, thus biasing the geographical representation and not incorporating representative samples for Europe.

Connectivity in marine species is assumed to be higher than in terrestrial species, due to lack of geographical barriers to gene flow expected in marine environments (Waples 1998). However, connectivity in the marine environment is often associated with oceanographic dynamics (e.g. oceanic currents, tides, bathymetry, upwelling systems, fronts, etc.), which can allow or prevent dispersal of pelagic forms and/or gametes (Palumbi 2004; Galarza et al. 2009). Oceanic fronts are narrow zones of enhanced

horizontal gradient of water parameters (e.g. temperature, salinity, nutrients, etc.), often persistent over seasons, and separate broader areas with different water masses of different vertical structure (stratification) (Belkin 2002). Different fronts have been described inside the three large marine ecosystems (LME) included in this study: Iberian Coastal LME (Spanish locations), Celtic-Biscay Shelf LME (Irish, French and English locations) and North Sea LME (Dutch and Danish locations) (Belkin and Cornillon 2007; Belkin et al. 2009). These fronts represent major barriers to planktonic dispersal, and they have strong influence on population structure of littoral organisms with both inshore (<2 km) and offshore (>2 km) pelagic larvae distribution (Galarza et al. 2009). Therefore, although the potential for migration is presumably high, fronts in the studied region could drive the differentiation among the three population groups. Thus, the Shelf-Slope Front of the Iberian coast (Atlantic and Cantabrian shores) is a thermohaline front which separates the saltier and mixed oceanic waters from the fresher and haline-stratified coastal waters (Fernandez et al. 1993). This front has been proposed as a retention mechanism for fish larvae (Fernandez et al. 1993; Gonzalez-Quiros et al. 2003, 2004; Rodriguez et al. 2009). This front could in effect isolate Spanish populations of flat oysters from others. The Irish Front, located in the Celtic Sea around the Saint George Channel and Bristol Channel, and the Ushant Front, located in front of French Brittany coast, are tidal mixing fronts which completely separate mixed waters columns over the coastal shallow zones and the stratified water column over the deeper oceanic zones (Belkin and Cornillon 2007). The Mid-Shelf Front, situated along Biscay Bay, western Irish coast and northern Scottish coast, is a shelf break front separating on-shelf coastal waters from off-shelf oceanic waters (Belkin and Cornillon 2007). These fronts are related with the distribution of nutrients and plankton in North-East Atlantic (O'Boyle and Raine 2007) and they would reduce the connectivity between the former locations and those from Ireland, England and France. Finally, the Frisian Front, located off the Dutch and German coasts, and the Danish Front, located in the western Jutland coast, are also tidal mixing fronts proposed to be the responsible of the differences in the macrofauna community structure (Kroencke et al. 2011), and they would further enforce the isolation of Dutch and Danish populations from the rest of European *O. edulis* beds (Belkin and Cornillon 2007). Interestingly, an oceanic salinity front caused by the freshwaters from the fjords and Skagerrak seas has been described along the Norwegian coast (Norwegian Coastal Current Front; Belkin et al. 2009), which could drive the flat oyster genetic differentiation previously reported for this region (Beaumont et al. 2006). Very similar population structure has been recently reported for the edible cockle (*Cerastoderma*

edule), with the presence of a southern group from Iberian coast to France, a northern group with populations from Denmark to Russia and a third central group composed by Irish, British and southern North Sea populations (Martinez et al. 2015). Previous studies explained the grouping of population by marine currents and historical processes including population expansions and secondary contacts of southern and northern groups in the English Channel zone, which is suggested as a hybrid/transition zone for other marine species including fish (Vandamme et al. 2014) and mollusc (Smietanka et al. 2004; Krakau et al. 2012). It is likely that glaciations have shaped the current distribution of population groups in the northern hemisphere favouring secondary contacts among groups previously differentiated (Hewitt 2000). Thus, high genetic variability is common in southern regions (e.g. southern European peninsulas including Iberian Peninsula), considered as glacial refugia, whereas rapid postglacial colonization resulted in northern groups with lower genetic diversity (Hewitt 1996, 2000; Larmuseau et al. 2010; Albaina et al. 2012). However, northern cryptic refugia have been previously described for marine species (Smietanka et al. 2004; Nikula et al. 2007; Krakau et al. 2012) associated with ecologically productive polynyas (areas of persistent open water), likely located between the Scottish and the Fennoscandia ice sheets (Krakau et al. 2012). Phylogeographic analyses with mtDNA in *O. edulis* showed the presence of ancestral related haplotypes in the most distant areas of its natural distribution (i.e. Norway and Black Sea; see Diaz-Almela et al. 2004). Thus, to speculate, the mtDNA results and those in the present study (genetic diversity and differentiation) could agree with the presence of two ancestral groups, a southern group composed by Spanish locations, a northern group by Dutch and Danish locations and a central one, where the two previous groups merged. This would be in accordance with the results reported by Martinez et al. (2015) for *C. edule* showing similar F_{ST} values to those described for *O. edulis* (F_{ST} values higher than 0.010 among sampling sites for different groups, see Supplementary Table 3). Neutral markers provide information about the effect of gene flow and genetic drift on genetic variability and its distribution. Thus, in spite of all results presented in our study suggest the presence of different genetic groups of *O. edulis* in the Atlantic area, further analyses based on genomic screening of a huge number of molecular markers (i.e. SNPs) should be performed to confirm the existence of local adaptation and thus the biological significance of the proposed groups.

Biogeographical barriers can also preclude the dispersal of individuals in marine ecosystems. Cape Finisterre (NW Iberian Peninsula) has been defined as a biogeographical barrier for some marine individuals including molluscs (Piñeira et al. 2008) and fish (Abaunza et al. 2008; Larmuseau et al.

2009), while it does not act as a barrier for gene flow in other species (e.g. seahorses, Lopez et al. 2015). The survey carried out at microgeographical scale in the Galician coasts (NW Spain), with locations situated northwards (SP-EOA, SP-OR, SP-FER, SP-PNTD) and southwards (SP-NOI, SP-PON) of this discontinuity, did not show population differentiation among locations within the region. This result indicates the potential presence of a single panmictic population unit (i.e. high natural gene flow) in the region apparently unaffected by the Cape Finisterre discontinuity. However, Beaumont et al. (2006) performed genetic studies along the Scottish coasts on a similar geographical scale as the Galician coast and suggested that genetic homogeneity could be the consequence of human-mediated transplantation. Therefore, anthropogenic causes for the lack of clear population structure cannot be ruled out as the cause for the homogeneity detected among samples from the Galician coast, and also in the other two proposed clusters.

Management and conservation of wild flat oyster beds

The main objective for species conservation is the preservation of viable wild populations. This is particularly important for farmed species as wild populations are the source of genetic variation of pinnacle importance for breeding programmes. Flat oysters have been reared for many centuries in Europe, and human-mediated transplantations are well recorded including transplantation of seeds from geographically close regions but also from more distant ones (Magennis et al. 1983; Sobolewska and Beaumont 2005; Beaumont et al. 2006). These transplantations would tend to genetically homogenize the wild oyster beds. However, our results show that genetic differentiation of wild flat oyster populations along Atlantic region is still present, despite human-mediated transplantation since ancient civilizations, which could impact mainly at regional level as suggested previously by Beaumont et al. (2006). The OSPAR convention defines five main regions inside the North-East Atlantic region based on their biogeographical characteristics and exploitation uses, and *O. edulis* is present in region II (Greater North Sea), region III (Celtic Sea) and region IV (Bay of Biscay and Iberian coast) (Fig. 1). Hence, the three suggested genetic clusters in the present study fit with OSPAR regions, although English locations were genetically clustered with Irish ones while they belong to two different OSPAR regions (region II and III, respectively).

Another important issue for the *O. edulis* conservation is the control of the parasite *Bonamia ostreae*. Bonamiosis has drastically reduced the production of *O. edulis* in Europe during the second half of the twentieth century (Lallias et al. 2007). Limited population differentiation at neutral molecular markers (i.e. microsatellite loci) has

been detected among the naïve (free of disease) Danish and Bonamia-affected Dutch oysters (pairwise F_{ST} values ranged from 0.0025 to 0.0034), and accordingly these two regions were clustered in a single genetic group. However, differences among genomic regions under selective pressure driven by *B. ostreae* infection cannot be ruled out. Differences in bonamiosis resistance have been described for individuals collected in different regions (Greece, Galicia and Ireland), and families from populations long exposed to the parasite are more tolerant (da Silva et al. 2005). Also, breeding programmes for resistance to bonamiosis have been successful, thus demonstrating genetic variation underlying tolerance to the parasite (Naciri-Graven et al. 1998; Culloty et al. 2004). It is clear that populations which have not been in contact yet with the parasite will be more susceptible than those exposed for a long time which could have developed some resistance. Thus, it is important to avoid transplantations of individuals (mainly adults, see Culloty and Mulcahy 1996) as naïve populations might be more sensitive to the disease. In addition, transplantations should be avoided in an effort to limit the spread of the disease to new areas. As suggested above, it is possible that genetic screening with a larger number of markers (e.g. SNP-based methods) could detect footprints of selection related to resistance to bonamiosis with potential use in breeding programmes and conservation.

Despite the high gene flow among regions, the significant genetic differentiation detected in the present study suggests that there are differentiated gene pools with possible potential for local adaptation in *O. edulis* which should be confirmed by the identification of footprints of selection at specific markers (i.e. outliers). Moreover, these gene pools have relatively large effective population sizes. Thus, the findings from our study suggest that transplantations among countries should be avoided and that, to improve the wild bed conditions/environments, habitat restoration should be the focus of *O. edulis* restoration. In addition, it is essential to control the presence of *B. ostreae* to avoid an increase of parasite dispersion to regions free of the disease (e.g. Danish populations) (Laing et al. 2006; Beck et al. 2011; Bromley et al. 2016).

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

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