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Population genetic structure of the European lobster (*Homarus gammarus*) in the Irish Sea and implications for the effectiveness of the first British marine protected area

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Abstract

Levels of self-recruitment within and connectivity among populations are key factors influencing marine population persistence and stock sustainability, as well as the effectiveness of spatially explicit management strategies such as Marine Protected Areas (MPAs). In the United Kingdom (UK), Lundy Island in the Bristol Channel was designated a No-Take Zone (NTZ) in 2003 and became the UK's first Marine Conservation Zone (MCZ) in 2009. This NTZ is expected to represent an additional resource for the sustainable management of the European lobster (*Homarus gammarus*) fishery. As the first step in a genetic monitoring program, this study aimed to investigate population genetic structure of lobster within and between the Irish Sea and Bristol Channel and in doing so to assess the functioning of the Lundy NTZ in the context of connectivity and other genetic parameters. Analysis of microsatellite data indicated that lobsters within the study area are genetically homogeneous and supports the view of a single panmictic population wherein the Lundy NTZ is highly connected. Levels of genetic variability were universally high with no evidence of differences for the NTZ. Furthermore, there was no evidence of recent genetic bottlenecks, and estimates of effective population sizes were infinitely large. The results suggest that if current management and breeding stock sizes are maintained genetic drift will not be strong enough to reduce neutral genetic diversity.

Keywords: Marine protected area; population genetics; sustainability; larval connectivity; recruitment; crustacean

1. Introduction

Spatial patterns of self-recruitment and connectivity are recognised as key factors shaping the dynamics of marine populations and how they respond to natural and/or human disturbances (Hastings & Botsford 2006). For harvested species, failure to identify independent (non-connected) population units can lead to local overfishing and ultimately, severe declines. Self-recruitment and connectivity also determine the efficacy of management strategies, such as marine protected areas, that are increasingly being implemented as tools to simultaneously achieve both fisheries management and biodiversity conservation objectives (McCook et al. 2009; Gaines et al. 2010; McCook et al. 2010). Specifically, the functioning of marine reserves is dependent on the degree to which MPA individuals contribute to populations within the MPA (Claudet et al. 2008; Fenberg et al. 2012), and through spillover/dispersal, to areas beyond reserve borders (Grüss et al. 2011a; Grüss et al. 2011b; Harrison et al. 2012). Connectivity thus influences the extent to which such MPAs may contribute recruits to fished areas, as well as other MPAs (Palumbi 2003).

In benthic-orientated marine species, which are often relatively sedentary as adults, connectivity is largely shaped by dispersal during a larval phase (Cowen et al. 2007); the sampling of which is logistically challenging. Combined with a generally poor understanding of the interactions between dispersal and oceanic features, the measurement of marine connectivity remains extremely difficult. In this sense, genetic tools are recognised as an extremely powerful resource to provide indirect estimates of connectivity, and as such to describe population dynamics and to predict, validate and quantify the ecological and economical success of MPAs (Christie et al. 2010b).

The European lobster, *Homarus gammarus*, is widely distributed in the northeast Atlantic, with its range extending from the Arctic Circle to Morocco, although it is not present in the Baltic Sea due to lowered salinity and temperature extremes (Triantafyllidis et

al. 2005). Population genetic studies of *H. gammarus* have been performed using microsatellite (Ferguson, unpublished data), mitochondrial DNA (mtDNA) (Triantafyllidis et al. 2005), and allozyme (Jørstad et al. 2005) markers but have to date focused on macrogeographical scales. While *H. gammarus* has a long pelagic larval duration offering the potential for extensive dispersal, genetic studies of other marine invertebrates indicate that local oceanography, and other physical and biological factors can contribute to fine scale genetic structuring and recruitment variability (Selkoe et al. 2006; Banks et al. 2007; Iacchei et al. 2013). Hence, finer scale genetic studies of *H. gammarus* are required to understand population structure at geographical scales more relevant to the species' fishery, especially in light of signatures of over-exploitation across Europe (Browne et al. 2001).

H. gammarus has been a socioeconomically important resource for Irish coastal communities for over a century (Browne et al. 2001). In this time there have been significant fluctuations in landings (>460%) and the relative importance of the fishery has changed with the development of ancillary crustacean fisheries (Browne et al. 2001). The species supports an important fishery in the Irish Sea where it is intensively harvested by both Irish and British fishers (Table S1). High levels of exploitation, by British fishers only, are also reported for the adjoining Bristol Channel (Table S1). A prominent feature of the Bristol Channel fishery is the marine protected area at Lundy Island (off the North Devon coast). This was first designated as a No-Take Zone (NTZ) in 2003 and became the United Kingdom's (UK) first Marine Conservation Zone (MCZ) in 2009.

The Irish Sea receives Atlantic water and influences from the Celtic Sea and St. Georges Channel in the south, and via the North Channel and Malin Shelf Sea in the North (Howarth 2005). The semi diurnal tides are the dominant physical process in the region with the North and South tidal streams meeting in a region just south of the Isle of Man, where they form a standing wave (Howarth 2005). The southern emanating tidal streams represent a

potential vector for northward larval dispersal, a likely mechanism linking the Bristol Channel and Irish Sea lobster populations. However, hydrodynamic modelling indicates patterns of stratification and residual flows that may significantly constrain larval recruitment while models of larval dispersal incorporating different behaviours (time of release and swimming) have reported substantially different patterns of predicted dispersal (http://www.susfish.com/index.php/workPackagesDetails/2). As such, empirical data are needed to assess patterns of connectivity within and between the Irish Sea and Bristol Channel, and in doing so to assess the connectivity of the Lundy NTZ.

Microsatellites have been shown to be capable of resolving fine scale population structure (McKeown et al. 2015) and were employed in this study to test the general hypothesis of a panmictic population of *H. gammarus* across the Irish Sea and Bristol Channel against alternatives of inter- and intra-regional structuring. Encapsulated within this was an assessment of the connectivity and other genetic parameters of the Lundy NTZ at local and regional scales. Studies such as this one will provide a baseline for future genetic monitoring of the lobster stock within the area and contribute to holistic assessments of the impact of small marine reserves on fisheries enhancement and sustainability.

2. Materials & Methods

2.1. Sample collection, DNA isolation and microsatellite genotyping

Samples were collected from lobsters at Lundy Island from within the NTZ (Lundy No-Take Zone, LNTZ) and outside the NTZ (Lundy Island Control Zone, LICZ), and from seven locations within the Irish Sea and Bristol Channel (Table 1, Figure 1). All samples consisted of mixed cohorts of adults.

Haemolymph was extracted from lobsters using a 2 ml Terumo syringe with a G23x25 mm needle (VWR International Ltd.) and preserved in absolute ethanol (1:8). DNA was extracted using the QIAGEN DNeasy Blood & Tissue Kit (QIAGEN, CA-USA) after an initial centrifugation step of 400 µl of the haemolymph/ethanol mixture for 5 minutes at 7000 x g in order to precipitate the cells into a pellet easily separated from the alcoholic fraction.

Twelve species-specific microsatellite loci (André & Knutsen 2010) were amplified in two multiplex PCR reactions. Amplification was carried out using the QIAGEN Multiplex PCR Kit (QIAGEN, CA-USA) in a final volume of 10 µl. This contained 5 µl of Multiplex Kit Buffer, 1 µl of genomic DNA (~100ng) and 0.2 µl of each forward and reverse primer for the specific multiplex reaction. The PCR cycle involved an initial denaturation step at 95 °C for 15 minutes, followed by 34 cycles of 45 seconds at 94 °C, 45 seconds at 59 °C and 45 seconds at 72 °C, and a final extension step at 72 °C for 45 minutes. Products were then run on an ABI 3730 Genetic Analyzer (Applied Biosystems) alongside a GS500LIZ size standard and alleles were inferred using GeneMapper 4.0 (Applied Biosystems).

2.2. Statistical analysis of microsatellite data

Genetic variation within samples was characterised using number of alleles (N_A), effective number of alleles (AEFF), allelic richness (AR; El Mousadik & Petit 1996), observed heterozygosity (H_o), and expected heterozygosity (H_e) (Nei 1978), calculated using GENALEX 6.2 (Peakall & Smouse 2006). Deviations from Hardy-Weinberg equilibrium (HWE) expectations and genotypic linkage equilibrium (LD) between pairs of loci were tested using exact tests (10 000 batches, 5000 iterations) in GENEPOP 3.3 (Raymond & Rousset 1995). Deviations from HWE were measured using F_{IS}, calculated according to Weir & Cockerham (1984) and tested for significance by 10 000 permutations in FSTAT 2.9.3

(Goudet 1995). Mean pairwise relatedness within samples was calculated using the relatedness estimator, r_{qg}, of Queller & Goodnight (1989) in GENALEX with associated 95% confidence intervals determined by 1000 bootstraps. The 95% confidence intervals for the expected range of r_{qg} under a panmictic model were calculated following 999 permutations of genotypes among samples.

Genetic differentiation between and among samples was assessed using pairwise and global (i) F_{ST} values, calculated in FSTAT with significance assessed by 10 000 permutations, and (ii) exact tests of allele frequency homogeneity performed in GENEPOP (10 000 batches 5000 iterations). The simulation method implemented in POWSIM (Ryman & Palm 2006) was used to estimate the sample size-dependent Type I and Type II error probabilities of exact tests. F_{ST} values were also calculated using the null allele correction method in FreeNA (Chapuis & Estoup 2007). The assumption of neutrality of the microsatellite loci was assessed using the FDIST outlier test (Beaumont & Nichols 1996) implemented in LOSITAN (Antao et al. 2008). Outlier tests were performed globally and between pairs of samples. F_{ST} matrices were visualised in a Multi-Dimensional Scaling plot constructed in R (R Core Team 2013) with the pcoa function. Genetic relationships were also visualised by a Principal Component Analysis (PCA) performed in PCAGEN (Goudet 1999). Mantel tests, implemented in the IBDWS software (Jensen et al. 2005) were used to test for correlation between pairwise F_{ST} and geographical distances between sample sites (i.e. isolation by distance). Geographical distances were calculated as the shortest sea distances between approximate centres of sampling locations using NETPAS 2.5. Genetic structure was also investigated without *a priori* sample information included using the Bayesian clustering analysis implemented in the program STRUCTURE (Pritchard et al. 2000; Falush et al. 2003, 2007). Following recommendations by Hubisz et al. (2009) analyses were

158 replicated for both the original ‘no locprior’ and new ‘with locprior’ models. Each run
159 consisted of a burn-in of 1 000 000 steps followed by 5 x 1 000 000 steps.

160 To investigate asymmetric dispersal patterns (source-sink recruitment) recent
161 migration rates were inferred using the Bayesian inference approach implemented in the
162 program BAYESASS 3.0.3 (Wilson & Rannala 2003). Simulations with a unique seed value
163 were run for 10 000 000 iterations with MCMC chains sampled every 1 000 iterations,
164 following an initial burn-in of 1 000 000 iterations. As suggested in the program
165 documentation, the five mixing parameters were adjusted to ensure acceptance rates between
166 20% and 60%, with ΔM, ΔA and ΔF set to 0.70. In order to examine convergence, the
167 posterior mean parameter estimates of multiple runs were compared.

168 Effective population sizes (N_e) were estimated using LDNE (Waples & Do 2008), in
169 which a random mating model was assumed. To test for recent effective population size
170 reductions, BOTTLENECK 1.2 (Piry et al. 1999) was utilised using 10 000 permutations for
171 the Infinite Allele (IAM) (Crow & Kimura 1970), Stepwise Mutation (SMM) (Ohta &
172 Kimura 1973) and Two-Phase Mutation (TPM) (Di Rienzo et al. 1994) models of
173 microsatellite evolution. Approximately 90% of microsatellite mutations are single step
174 (Garza & Williamson 2001), thus the parameters of the TPM model were set to run at 90% of
175 single step mutations with a variance of 10 among multiple steps. The Wilcoxon test was
176 used to determine whether any of the sample sites show an excess of heterozygosity, which is
177 expected after a severe bottleneck (Cornuet & Luikart 1996). In addition, the graphical mode-
178 shift test was incorporated to detect shifts from the normal L-shaped distribution of allele
179 frequencies that are expected at equilibrium (Luikart et al. 1998). A second approach to
180 assess for evidence of a population bottleneck was the *M*-ratio between the total number of
181 alleles (*k*) and the overall range in allele size (*r*) (Garza & Williamson 2001). *M* was
182 calculated in ARLEQUIN 3.5 (Excoffier & Lischer 2010) by taking the mean population

183 value from the modified Garza-Williamson (GW) index computation. The modification of the
184 GW index avoids a division by zero when a gene sample is fixed for a single allele (Excoffier
185 et al. 2005). Values of $M < 0.68$ were considered as a sign of a recent population bottleneck
186 (Garza & Williamson 2001), as described in Coscia et al. (2012).

187

188 **3. Results**

189 The total number of alleles per locus ranged from seven to 17 (mean = 10.17) and
190 levels of genetic variability were highly similar among all samples (Table 1; Table S2; Figure
191 S1). Three locus-pairings were found to be in global linkage disequilibrium: HGD106 &
192 HGC131b (P = 0.025); HGC129 & HGC118 (P = 0.019); and HGD111 & HGC103 (P =
193 0.042). However, these test results became non-significant after Bonferroni correction.
194 Furthermore, significant global test results were due to significant results occurring only in
195 one or two samples in each case (HGD106 & HGC131b locus-pairing: WEX, P = 0.003;
196 HGC129 & HGC118 locus-pairing: LNTZ, P = 0.043, and; HGD111 & HGC103 locus-
197 pairing: SW, P = 0.002; NW, P = 0.016) and became non-significant upon omission of these
198 samples. All deviations from HWE were due to heterozygote deficits. Eight out of a total 108
199 individual locus/sample tests exhibited deviations from HWE (at $P < 0.05$), with two
200 remaining significant after Bonferroni correction: LNTZ and LICZ at locus HGA8 ($P <$
201 0.0005). Multilocus deviations from HWE were reported for the Waterford (WF) and North
202 Dublin (ND) samples from Ireland and both Lundy samples from the Bristol Channel (LNTZ,
203 LICZ) with LNTZ exhibiting the largest F_{IS} value (Table 1). In all cases these were due to
204 single locus deviations occurring at HGA8, and became non-significant after omission of this
205 locus. Mean kinship values were within ranges predicted for a panmictic model but there
206 were significant differences between samples (Figure 2).

A global exact test of genic homogeneity was non-significant (P = 0.727) as were all pairwise comparisons except that between LNTZ and SW (P < 0.05) (Table 2). Simulation analysis performed in POWSIM indicated that the locus polymorphism and sample sizes used in this study would have conferred sufficient power for tests to detect even low levels of 'true' genetic structuring (estimated probability of 0.99 that the data would identify differentiation at true F_{ST} = 0.01) while retaining a low Type I error probability (P = 0.04). Similarly, global F_{ST} (-0.000; 95% CI: -0.002 – 0.001) and all pairwise F_{ST}s were non-significant for both unedited and null allele corrected data sets (Table S3). LOSITAN confirmed the neutrality of the 12 microsatellite markers that were used, with none being selected as candidates for balancing or positive selection in global or pairwise tests. Visualisation of pairwise F_{ST} using MDS revealed no geographic pattern (Figure 3) and there was not significant IBD (r = 0.057, P = 0.35). Bayesian clustering analysis did not provide evidence of population structuring, with all runs unanimously supporting a model of K=1. Inconsistencies between the posterior mean parameter estimates for the BAYEASS simulations indicated there was insufficient information in the data to accurately infer recent migration rates among LNTZ, SW, DEV, and WF, WEX. Estimates of N_e suggest that the sampled populations were very large, with upper 95% confidence intervals including infinity (Table 1). BOTTLENECK reported significant results under the IAM for the SW (P = 0.032) and DEV (P = 0.017) samples but all tests assuming a TPM or SMM were not significant. Allele frequency distributions revealed no mode shifts for any sample (i.e. standard L shapes detected). The *M*-ratio values for all samples suggested historical population size reductions.

4. Discussion

Microsatellite markers represent powerful tools to investigate demographic processes that may be beyond the resolution of other genetic marker types (Shaw et al. 2004). This

study is the first to employ microsatellites to look at fine scale population processes in *H. gammarus*. Simulation analysis indicated that marker polymorphism and sample sizes in this study conferred considerable power to detect even low levels of genetic differentiation between samples. Global and pairwise (F_{ST} and exact) tests provided no evidence of significant differentiation among samples. This pattern of genetic homogeneity was also replicated for analysis of sex partitioned samples. For many marine species, estimates of genetic structure may be compromised by adult dispersal (resulting in mechanical mixing of differentiated populations) (Nielsen et al. 2004). However, as *H. gammarus* adults are largely sedentary (Jensen et al. 1993; Bannister et al. 1994; Smith et al. 2001; Moland et al. 2011; Øresland & Ulmestrand 2013), postlarval dispersal is unlikely to influence results. Furthermore, Bayesian clustering analysis provided no evidence of genetic structuring, a pattern which is typical of lobsters (Tolley et al. 2005; García-Rodríguez & Perez-Enriquez 2008; Naro-Maciel et al. 2011) except where gene flow is restricted by conspicuous physical drivers (e.g. oceanography; geographic barriers) (Gopal et al. 2006; Palero et al. 2008). The lack of population structure indicates high levels of gene flow and connectivity within and between the Irish Sea and Bristol Channel.

The overall genetic homogeneity is also consistent with connectivity of the Lundy NTZ. Samples collected outside of the Lundy NTZ reported similar levels of genetic diversity to the Lundy NTZ sample, which is also compatible with a lack of isolation for the Lundy NTZ. Calò et al. (2016) reported similar levels of genetic diversity for protected and unprotected areas in the saddled sea bream (*Oblada melanura*). This pattern differs from the study including two MPAs in the western Mediterranean by Pérez-Ruzafa et al. (2006) that reported higher values of total and standardised allelic richness in protected populations of *Diplodus sargus* than in unprotected ones.

256 The similar levels of genetic diversity between MPA and non-MPA sites observed for
257 *H. gammarus* may be a combination of (i) the high level of connectivity throughout the
258 studied area and (ii) a lack of fishery induced genetic erosion. The significant *M*-ratio test
259 results obtained for all *H. gammarus* samples likely reflect ancestral bottlenecks common to
260 all populations within the study area (Girod et al. 2011). Similar genetic signatures of
261 ancestral population size changes associated with historical climate change events such as the
262 Pleistocene glaciations have been detected in a range of lobster species (Gopal et al. 2006;
263 García-Rodríguez & Perez-Enriquez 2008; Naro-Maciel et al. 2011). No compelling evidence
264 of more recent bottlenecks was discovered in any *H. gammarus* sample. Although the Gower
265 (SW) and Ilfracombe (DEV) samples revealed significant bottleneck signatures using the
266 Wilcoxon sign rank test, this is not considered as strong evidence for a bottleneck, as test
267 results were only significant under the IAM, a mutation model deemed unlikely for
268 microsatellites (Piry et al. 1999), and it is recommended to use the SMM to be statistically
269 conservative when testing for recent bottlenecks (Luikart & Cornuet 1998). This was further
270 confirmed by the lack of L-mode shifts, intrasample relatedness values within expected
271 ranges for a panmictic population, and estimates of N_e which included infinity. Levels of
272 intrasample variation (H_o, H_e, N_A) were also comparable to values reported for putative 'non-
273 bottlenecked' populations sampled from other regions (Ferguson, unpublished data)
274 indicating that *H. gammarus* has not undergone any recent genetic erosion due to fishing
275 activities within the Irish Sea or Bristol Channel and retains a high level of genetic variation.

276 An important consideration is that the high background levels of genetic diversity
277 may make any MPA associated increase in diversity difficult to detect. Moreover, any genetic
278 changes, positive or negative, associated with the NTZ may not be detectable for a number of
279 generations and thus may not be apparent due to its recent establishment. As such, this study
280 must form part of an ongoing genetic monitoring program.

In this study significant heterozygote deficits were reported for the Irish Waterford and North Dublin samples and the two Lundy samples, with the Lundy NTZ exhibiting the largest deficit. In all cases these deficits were driven by a single locus (HGA8), indicating a null allele effect. However, in light of the low level of genetic structure throughout the studied area, the restriction of such heterozygote deficits to particular samples, with the largest deficit reported for the NTZ, suggests there may be some biological component. Significant heterozygote deficits are a common feature among marine invertebrates with biological drivers including inbreeding, selection and Wahlund effects (Addison & Hart 2005). Inbreeding can be discounted as values of relatedness for all samples are in line with expectations of panmixia and provide no evidence that closely related individuals have been sampled. Selection against heterozygotes has rarely been documented (Teske et al. 2013) and outlier tests reported no evidence of selection effects at the employed loci. Based on the low level of genetic structure throughout the studied region spatial Wahlund effects due to the sampling of mixtures of individuals derived from genetically differentiated populations can also be discounted. However, for many highly fecund marine species with pelagic larval stages, large variances in reproductive success occur among individuals (sweepstakes recruitment) such that in any reproductive event only a small fraction of adults contributes to the next generation (Hedgecock 1994). This can generate genetic differences within and between cohorts (cohort Wahlund) (Ruzzante et al. 1996) without restricted gene flow where recruitment is temporally or spatially variable (Johnson et al. 1993). By a process of elimination, sweepstakes effects as a component of overall localised recruitment stochasticity, represents the most plausible biological mechanism for generating the reported heterozygote deficits. Palero et al. (2011) suggested that for the lobster *Palinurus elephas*, the biased exploitation of large individuals led to a reduction in the number of large females and a consequent reduction in variance in reproductive success. In the case of the Lundy NTZ, the

306 predicted persistence of larger females (Wootton et al. 2012) may increase the scope for
307 variance in reproductive success. Furthermore, a paternity study in the American lobster, *H.*
308 *americanus*, reported evidence of high levels of multiple paternity at exploited sites but single
309 paternity at the only unexploited site (Gosselin et al. 2005). Similar fishery associated
310 reduction in multiple paternity at the Lundy NTZ would serve to increase variance in
311 reproductive success compared to other multiple paternity areas (McKeown & Shaw 2008).
312 Confirmation and identification of any such NTZ effects is vital and will require comparative
313 analysis of other species and ideally comparisons of intraspecific patterns associated with
314 other NTZs.

315 In conclusion, the data indicate that within the studied area, *H. gammarus*, including
316 those within the NTZ, belong to a single panmictic population. Comparable levels of genetic
317 diversity within the Irish Sea and with other regions indicate that the species retains high
318 levels of neutral genetic diversity despite being heavily exploited. Spatial genetic
319 homogeneity and large effective population size estimates suggest that if current breeding
320 stock sizes are maintained, loss of neutral genetic variation within the Irish Sea and Bristol
321 Channel is unlikely. The high connectivity throughout the studied area emphasises the need
322 for cooperation between British and Irish fishery managers and also has implications for the
323 spatial distribution of future MPAs and the establishment of a MPA connected network
324 (Larson & Julian 1999). Future research aimed at describing even finer elements of
325 demographic connectivity would benefit from employing genome wide SNP analyses. These
326 offer the potential to assay a huge number of loci for use in isolation-with-migration models,
327 and may reveal asymmetric gene flow dynamics (Wang & Hey 2010; Marko & Hart 2011)
328 that were beyond the resolution of the current data set, as indicated by the simulations
329 performed for BAYEASS. Additionally, direct estimates of dispersal could be obtained

through genetic parentage analysis of new recruits (Jones et al. 2005; Planes et al. 2009; Christie et al. 2010a; Saenz-Agudelo et al. 2011; Buston et al. 2012; D'Aloia et al. 2013).

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Tables

Table 1. Sample information and summary statistics of *Homarus gammarus* in the Irish Sea: H_e expected heterozygosity, H_o observed heterozygosity, N_A number of alleles, AEFF effective number of alleles, A_R allelic richness, F_{IS} inbreeding coefficient, and N_e estimates of effective population size with 95% confidence intervals (parametric). Significant values of F_{IS} are denoted by * ($P < 0.05$) or *** ($P<0.001$).

Sampling Location	Code	Coordinates	N	H_e	H_o	N_A	AEFF	A_R	F_{IS}	N_e (95% CI)
Howth, Dublin	ND	53.469° N, 6.084° W	36	0.69	0.68	7.25	3.44	6.99	0.016	-272.7 (474.0 - ∞)
Carne, Wexford	WEX	52.184° N, 6.302° W	29	0.67	0.64	7.00	3.42	7.00	0.037	677.7 (98.3 - ∞)
Dunmore East, Waterford	WF	52.085° N, 7.033° W	48	0.68	0.64	7.92	3.59	7.24	0.050*	-768.4 (400.7 - ∞)
North Llŷn Peninsula, Gwynedd	NW	52.806° N, 4.823° W	40	0.67	0.64	7.92	3.47	7.30	0.049*	2407.4 (187.2 - ∞)
Aberystwyth, Ceredigion	CB	52.415° N, 4.236° W	44	0.67	0.66	7.58	3.34	7.07	0.012	-1234.9 (253.3 - ∞)

Gower, Swansea	SW	51.550° N, 4.144° W	48	0.68	0.69	7.83	3.62	7.15	-0.017	-924.1 (378.0 - ∞)
Lundy Island (outside NTZ)	LICZ	51.205° N, 4.682° W	44	0.68	0.65	8.00	3.66	7.42	0.046*	1252.1 (175.6 - ∞)
Lundy Island NTZ	LNTZ	51.189° N, 4.649° W	48	0.69	0.64	7.75	3.61	7.17	0.072***	-406.8 (642.2 - ∞)
Ilfracombe, Devon	DEV	51.228° N, 4.125° W	48	0.69	0.68	7.50	3.65	6.97	0.014	-916.0 (363.0 - ∞)

566

567

568 **Table 2.** Genetic differentiation of *Homarus gammarus* samples from the Irish Sea and
569 Bristol Channel. F_{ST} pairwise comparisons (lower diagonal) and exact G tests (upper
570 diagonal). * denotes significant G-tests ($P < 0.05$).

	LNTZ	LICZ	WF	CB	ND	WEX	SW	NW	DEV
LNTZ		21.057	23.444	25.968	24.981	19.784	36.454*	24.002	31.472
LICZ	-0.002		20.833	26.380	23.484	21.954	20.792	17.966	17.061
WF	0.002	-0.002		23.220	27.522	23.410	25.288	27.539	17.068
CB	0.000	0.001	0.002		30.277	22.052	23.771	15.187	23.773
ND	-0.002	-0.001	0.004	-0.001		23.467	24.216	18.863	24.525
WEX	-0.001	0.001	0.004	0.000	0.003		24.576	18.967	23.904
SW	0.001	-0.002	0.000	-0.001	0.000	0.001		14.771	17.262
NW	0.000	-0.002	0.004	-0.004	-0.003	0.001	-0.002		11.897
DEV	0.000	-0.001	0.000	-0.001	-0.002	0.003	-0.003	-0.004	

571

572

573 **Figure legends**

574 **Figure 1.** Sampling locations of the European lobster, *Homarus gammarus*, in the Irish Sea,
575 highlighting the marine protected (white circle) and non-protected (black circle) areas.

576 **Figure 2.** Mean within-sample pairwise relatedness, r_{qg}, and associated 95% confidence
577 intervals derived from bootstrap resampling. Grey bars are the 95% upper and lower expected
578 values for a null distribution generated from 999 permutations of data from all samples.

579 **Figure 3.** MDS plot of pairwise F_{ST} values of *Homarus gammarus* in the Irish Sea.

580

Figure 1

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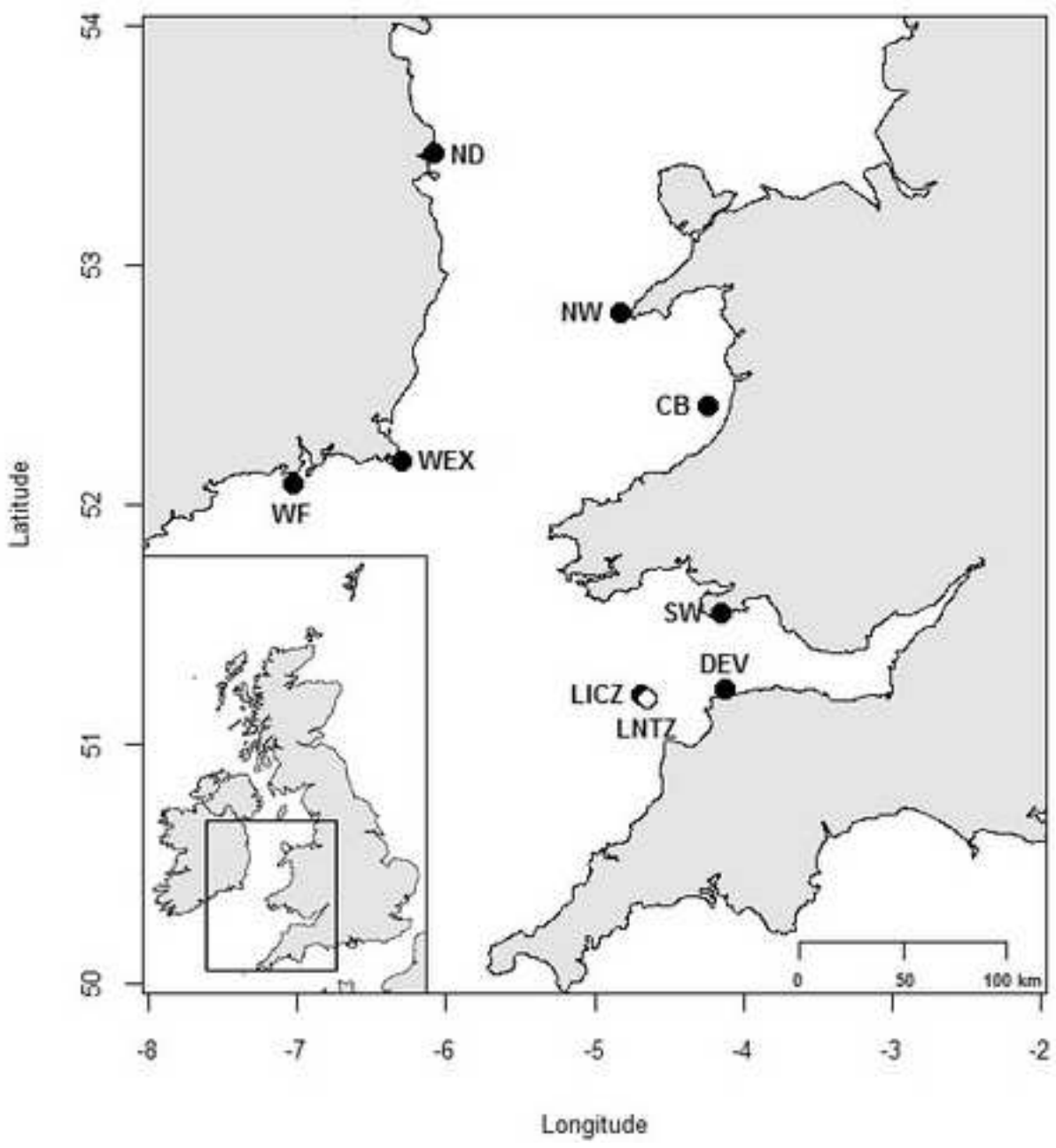

Figure 2

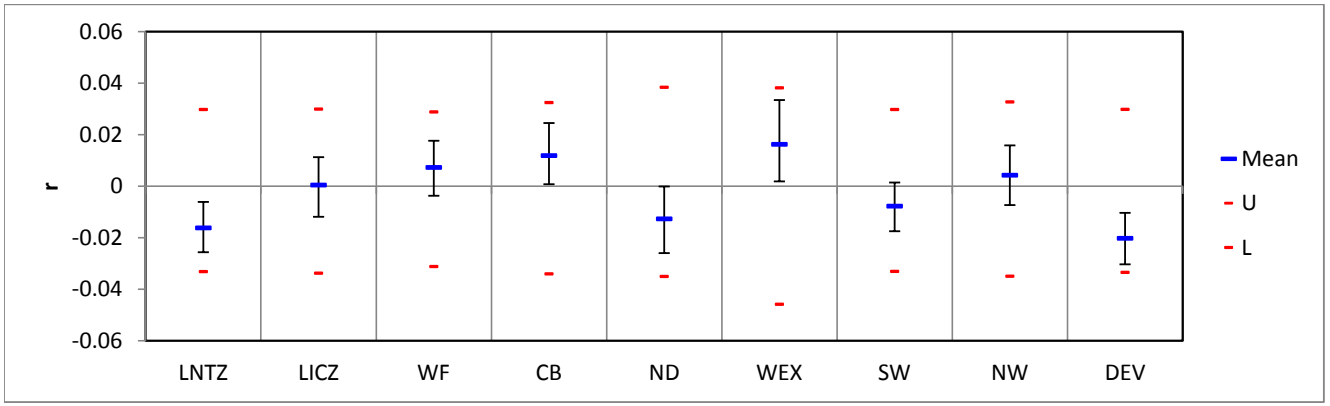
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Figure 3

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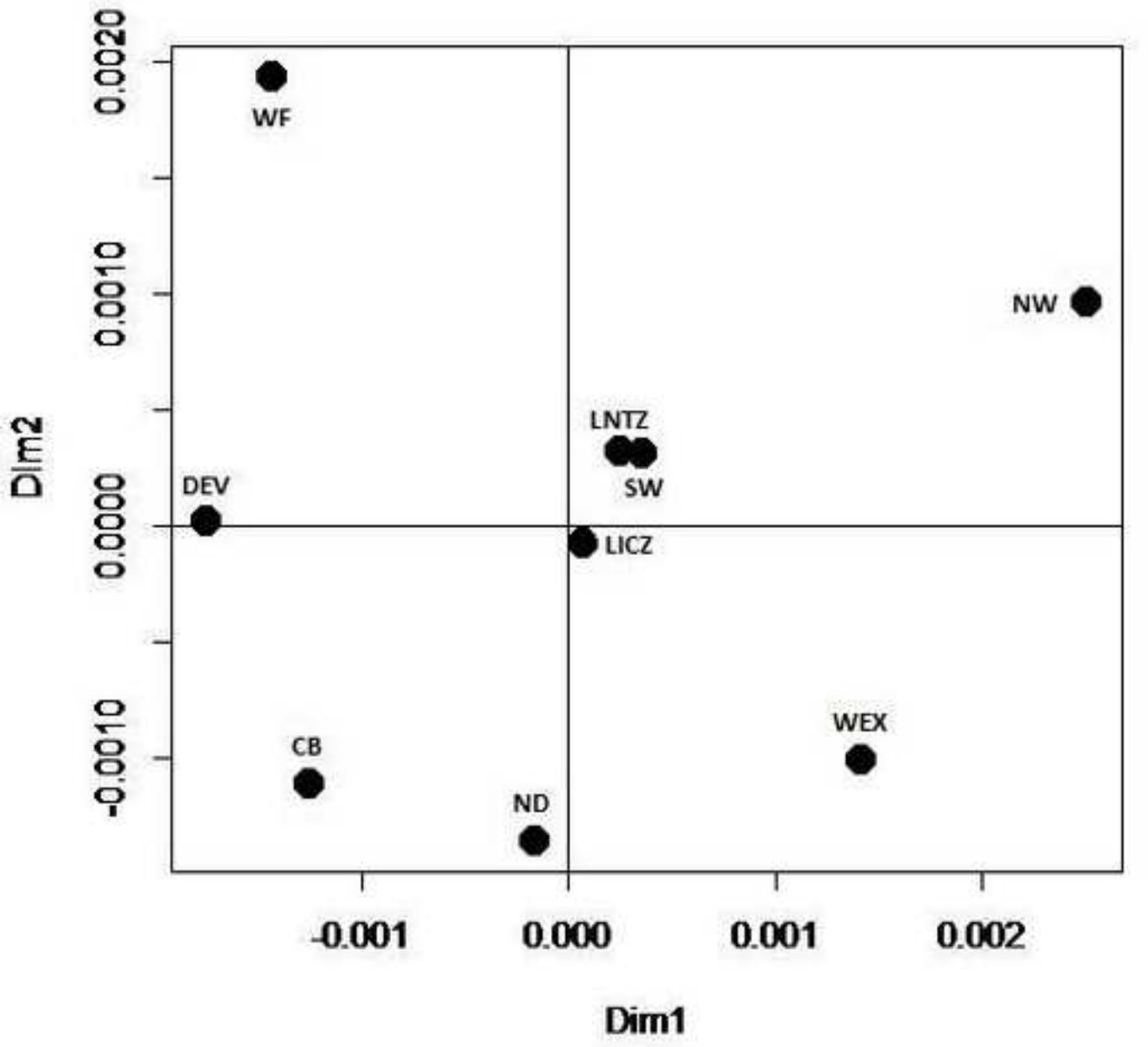

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