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Author for correspondence:
Joseph E. Ironside, E-mail: jei@aber.ac.uk

Genetic variation in Irish Sea brown crab (*Cancer pagurus* L.): implications for local and regional management

Niall J. McKeown[1], Hayley V. Watson[1], Ilaria Coscia[2], Emma Wootton[3] and Joseph E. Ironside[1]

[1]Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University, Aberystwyth SY23 3DA, UK; [2]School of Environment and Life Sciences, University of Salford, Salford M5 4WX, UK and [3]Department of Biosciences, Swansea University, Swansea, West Glamorgan SA2 8PP, UK

Abstract

Understanding demographic processes over multiple spatial scales is vital for the optimization of conservation/management strategies, particularly for commercially harvested taxa such as the brown crab (*Cancer pagurus* L). Brown crab population genetic structure was investigated at (i) a local scale within the Irish Sea, which included comparisons with the Lundy No Take Zone (NTZ) and (ii) across the NE Atlantic. The results indicate that the brown crab does not exhibit strong spatial structure either within the Irish Sea or at the regional level, suggesting high gene flow within and among the Irish Sea, English Channel and North Sea. Comparisons between the Lundy NTZ and harvested areas revealed similarly high levels of genetic diversity. An intriguing result was that the Lundy NTZ sample exhibited a degree of genetic patchiness (ephemeral geographically unpatterned differentiation) which may indicate elevated recruitment skews within the NTZ. Overall, the results support the view that brown crabs within the sampled area belong to a single genetically panmictic stock and that if breeding stock sizes are maintained genetic drift will not be strong enough to reduce neutral genetic diversity. The highly connected nature of this species requires international cooperation for sustainable management, an important component of which will be the application of more powerful population genomic approaches to assess finer scale aspects of stock structure as well drivers of genetic patchiness reported for the species. This is a timely consideration in light of potential future misalignments between biological and geopolitical stock boundaries in the Irish Sea following Brexit.

Introduction

Levels of self-recruitment within and connectivity among populations are key factors influencing marine population persistence and stock sustainability (Hastings & Botsford, 2006). Understanding these processes is therefore crucial for spatially explicit management strategies such as Marine Protected Areas (MPAs) (Palumbi, 2003). Population genetic studies of crustacean species have revealed that while some species appear to be almost panmictic over large geographic areas (Domingues *et al.*, 2010; Pampoulie *et al.*, 2011), others display extensive population structure (Babbucci *et al.*, 2010; Jorde *et al.*, 2015). Failure to identify discrete population units and/or mismatch between geopolitical and biological stocks may compromise fishery sustainability (Waples *et al.*, 2008; Reiss *et al.*, 2009). Species-specific genetic assessments over multiple spatial scales are therefore necessary for the optimization of spatial conservation/management strategies (Almany *et al.*, 2009).

Genetic studies of crustaceans have typically focused on taxa with sedentary adults (Jorde *et al.*, 2015). In this context the brown crab, *Cancer pagurus* (L.), occurring continuously in shallow shelf waters of the NE Atlantic from the Lofoten Islands (Norway) to Morocco (Bennett, 1995) and supporting one of the most important commercial European fisheries, represents an interesting candidate for investigation as both larval and adult stages have substantial dispersal potential. Adults are described as benthic and mobile, but there are pronounced dispersal differences between the sexes. Males are largely resident, making short random movements within small territories, while females migrate significantly longer distances, and more frequently, than males (Edwards, 1979; Bennett & Brown, 1983; Latrouite & Le Foll, 1989; Ungfors *et al.*, 2007). In the English Channel, female migrations of up to 200 nautical miles have been reported with some crabs achieving a mean speed of 1.07–1.62 nautical miles per day (Pawson, 1995). The pelagic larval stage lasts for approximately three months (Eaton *et al.*, 2003; Weiss *et al.*, 2009; Hunter *et al.*, 2013) and while little is known about the ecology of juveniles they are rarely caught in offshore waters, suggesting that adult crabs only move to deeper water as they grow and reach maturity. Tagging studies have revealed that adult female migrations are consistently against prevailing currents (Ungfors *et al.*, 2007; Hunter *et al.*, 2013). As the larvae are poor swimmers likely to passively drift while entrained in currents, it has been suggested that contranatent female migrations are a spawning behaviour aimed at facilitating return to areas of maternal origin. Even in the

absence of additional extrinsic factors, the seemingly opposing dispersal of females and larvae is expected to limit 'life-time dispersal' and may thus influence spatial patterns of recruitment and structuring of reproductive populations.

Previous genetic studies of brown crab are consistent with connectivity over large geographic areas. Ungfors *et al.* (2009), using microsatellite markers, reported no significant genetic differentiation among samples spanning 1300 km of waterway distance within the Norwegian Sea, Skagerrak and Kattegat. In a more geographically extensive study, using the same microsatellite loci, McKeown *et al.* (2017) described evidence of broad scale connectivity throughout the Celtic Sea, English Channel, North Sea and Scandinavian waters. Against this background of gene flow a number of samples exhibited 'chaotic genetic patchiness' (*sensu* Hedgecock, 1994) which was interpreted as being driven by a combination of spatio-temporal variation in recruitment and larval retention associated with coastline features, specifically bays and inshore areas. While such processes may be irrelevant over longer (i.e. evolutionary) timescales they may significantly impact stocks on timescales of interest to fishery stakeholders and may both decrease resilience of local stocks to fishing and increase unpredictability in recovery (Kuparinen *et al.*, 2014). They also highlighted how even in the absence of population isolation, crab management may need to be tailored at local levels to physical and biological drivers of such recruitment variability.

The Irish Sea supports a socioeconomically important fishery exploited by Irish and British fishers. The region also contains the UK's first Marine Conservation Zone (MCZ) located at Lundy Island in the Bristol Channel off the North Devon coast. The Irish Sea has been poorly represented in population genetic studies of brown crab to date. To address this knowledge gap, a primary objective of this research was to assess population genetic structuring within the Irish Sea and interpret such patterns in the context of connectivity and spatial recruitment patterns. The inclusion of samples from within the Lundy No-Take Zone (NTZ) also permitted analysis of the functioning of the MPA. As the Lundy NTZ is located in what might be considered a retentive environment, the urgency of such information is highlighted by the findings of McKeown *et al.* (2017) which suggested that such habitats may be associated with elevated recruitment stochasticity. In addition, Watson *et al.* (2016) found signatures of elevated recruitment variability within the Lundy NTZ for European lobster (*Homarus gammarus*). A second objective was to investigate the connectivity of the Irish Sea with other regions. This was obtained by integrating the data from McKeown *et al.* (2017) for loci common to both studies. The outcomes of this study provide useful information on (i) the spatial scale that should be considered for management of the brown crab resource and (ii) by comparison with a previous lobster study, the functioning and effectiveness of the Lundy NTZ for crustacean species with different life histories.

Materials and methods

Sample collection, DNA isolation and microsatellite genotyping

Haemolymph was collected from a total of 514 crabs at 13 locations within the Irish Sea (Table 1, Figure 1A). Sampling locations were along the coasts of Wales (West Anglesey (AW), Menai Straits (NW), Aberystwyth (CB), Oxwich (OX), Mumbles (M) and the Gower (SW)) and the Republic of Ireland (Howth (ND), Dun Laoghaire (SD), Carne (WEX) and Dunmore East (WF)). Samples were also collected at Lundy Island in the Bristol Channel (Lundy NTZ (LNTZ), outside the NTZ in May (LIA) and in June (LIB)). Haemolymph was collected from commercially caught individuals, hence above the 130 mm Minimum Landing Size (MLS) for the Irish Sea region (SEAFISH 2009), except for NW, OX and M where juveniles were sampled. DNA was extracted using either the QIAGEN DNeasy Blood & Tissue Kit (QIAGEN, CA-USA) or the CHELEX 100 (Bio-Rad, CA-USA) protocol (Walsh *et al.*, 1991). Individuals were then genotyped at 11 species-specific microsatellite loci developed by McKeown & Shaw (2008*a*) which were amplified in a single multiplex polymerase chain reaction (PCR). PCRs were carried out using a QIAGEN Multiplex PCR Kit (QIAGEN, CA-USA) in a final volume of 15 µl, containing 7.5 µl of Multiplex Kit Buffer and 1 µl of genomic DNA. Primer volumes varied: 0.35 µl Cpag-4C1; 0.3 µl Cpag-1B9, Cpag-3A2, Cpag-3D7, Cpag-4 and Cpag-5D8; 0.25 µl Cpag-38; 0.2 µl Cpag-1C8, Cpag-6C4B and Cpag-15; and 0.1 µl Cpag-2A5B. The PCR cycle involved an initial denaturation step at 95°C for 15 min, followed by 34 cycles of 45 s at 94°C, 45 s at 55°C and 45 s at 72°C, and a final extension step at 72°C for 45 min. PCR amplicons were separated using an ABI 3730 Genetic Analyzer (Applied Biosystems) and genotyped using GeneMapper 4.0 (Applied Biosystems).

To assess regional patterns comparative analysis of the Irish sea samples with samples from a wider geographic range across the NE Atlantic (Supplementary Table 1; Figure 1B), specifically the Celtic Sea, English Channel and North Sea including Scandinavian waters (McKeown *et al.*, 2017), was performed using genotypic data for the 8 loci common to both studies (Cpag-5D8, Cpag-4, Cpag-6C4B, Cpag-3A2, Cpag-1B9, Cpag-3D7, Cpag-2A5B and Cpag-15). To ensure compatibility of genotypes between studies a subset of individuals from McKeown *et al.* (2017) was re-genotyped using the protocols used for this study.

Statistical analysis

Genetic variation within samples was characterized using number of alleles (N_A), allelic richness (A_R; El Mousadik & Petit, 1996), observed heterozygosity (H_O) and expected heterozygosity (H_E) (Nei, 1978), all calculated using GENALEX 6.2 (Peakall & Smouse, 2006). Genotype frequency conformance to Hardy–Weinberg equilibrium (HWE) expectations and genotypic linkage equilibrium 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).

Genetic differentiation between and among samples was assessed using pairwise and global (i) F_{ST} values with significance assessed by 10,000 permutations and (ii) exact tests of allele frequency homogeneity, all performed in ARLEQUIN 3.5 (Excoffier *et al.*, 2010). 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. The assumption of neutrality of the microsatellite loci was assessed using the FDIST outlier test implemented in LOSITAN (Antao *et al.*, 2008). F_{ST} matrices were visualized using principal coordinate analysis in GENALEX. Mantel tests, implemented in GENALEX were used to test for correlation between pairwise F_{ST} and geographic distances between sample sites (i.e. isolation by distance (IBD)). Geographic distances were calculated as the shortest sea distances between approximate centres of sampling locations. Hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) was performed in ARLEQUIN to partition genetic variance among groups of samples (F_{CT}) and among samples within groups (F_{SC}) with significance levels of F_{CT} and F_{SC} tested using 1000 permutations. Genetic structure was also investigated without *a priori* sample

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Table 1. Brown crab sample information, including sample code, geographic coordinates, sampling time and sample size

Sample location	Coordinates	Code	Collection date/sample N	*Na*	*Ar*	H_o	H_e	F_{IS}
West Anglesey	53.213°N 4.606°W	AW	April–10 15	5.73	5.73	0.55	0.56	0.04
Cardigan Bay	52.415°N 4.236°W	CB	October-10 48	8.73	6.34	0.59	0.61	0.04
Lundy Island (outside NTZ) A	51.205°N 4.682°W	LIA	May-10 47	9.36	6.67	0.55	0.63	0.14*
Lundy Island (outside NTZ) B	51.205°N 4.682°W	LIB	June-10 22	7.64	6.54	0.58	0.59	0.03
Lundy Island NTZ	51.189°N 4.649°W	LNTZ	May-10 33	7.64	5.99	0.53	0.60	0.13*
Menai Straits	53.220°N 4.163°W	NW	June-11 48	8.73	6.19	0.58	0.61	0.06
Gower	51.550°N 4.144°W	SW	June-10 16	7.00	6.80	0.61	0.60	0.01
North Dublin (Howth)	53.469°N 6.084°W	ND	July-11 48	9.09	6.42	0.59	0.61	0.04
South Dublin (Dun Laoighre)	53.217°N 6.085°W	SD	July-11 48	9.09	6.33	0.57	0.58	0.03
Waterford (Dunmore East)	52.085°N 7.033°W	WF	July-11 47	8.73	5.97	0.58	0.59	0.04
Wexford	52.184°N 6.302°W	WEX	July-11 46	8.18	6.24	0.59	0.62	0.06
Mumbles	51.570°N 3.980°W	M	February + June 11 48	8.09	5.97	0.58	0.59	0.03
Oxwich	51.566°N 4.147°W	OX	June-11 48	9.27	6.51	0.60	0.61	0.02

Multilocus (mean) genetic variability measures: *Na* (allele number); *Ar* (allele richness); H_O (observed heterozygosity); H_E (expected heterozygosity); F_{IS} (standardized genetic variance within samples. * denotes significant deviations from HWE expectations.

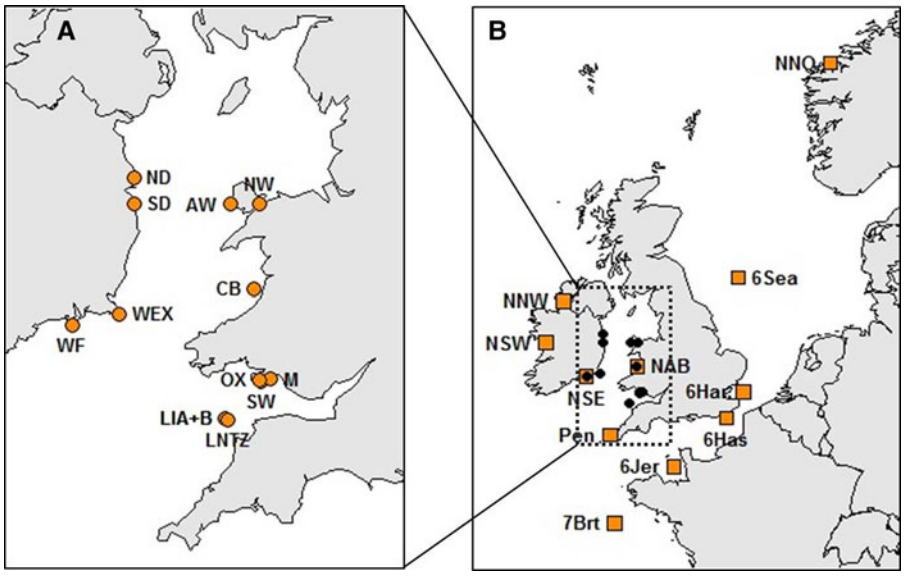

Fig. 1. Brown crab sample sites (see Table 1 and Supplementary Table 1 for details). (A) Irish Sea and Bristol Channel samples analysed for 11 microsatellite loci as part of new genotyping from this study. (B) Samples spanning the NE Atlantic for which data for 8 loci were obtained from McKeown *et al.* (2017) (amber boxes) overlaid upon the Irish Sea/Bristol temporal replicates (black dots).

information included using the Bayesian clustering analysis implemented in the program STRUCTURE (Pritchard *et al.*, 2000). Following recommendations by Hubisz *et al.* (2009) analyses were replicated for both the original 'no locprior' and new 'with locprior' models. Each run consisted of a burn-in of 10^6 steps followed by 5×10^6 steps. Randomization procedures in FSTAT were used to detect significant differences in heterozygosity, A_R, F_{IS}, F_{ST} and relatedness among user defined groups of samples following 10,000 permutations.

Results

Genetic diversity among Irish Sea crab

The total number of alleles range from 5 to 30 (average 12.8). Levels of genetic variability were very similar among samples (Table 1). All loci were polymorphic in each sample with the exception of Cpag1C8 (7 alleles overall) which exhibited no variation in 5 samples (AW, CB, LIB, LNTZ, M). No significant linkage disequilibrium was detected between any locus pair in tests

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Table 2. Pairwise F_{ST} estimates between all samples

	AW	CB	LIA	LIB	LNTZ	NW	SW	ND	SD	WF	Wex	M	OX
CB	0.010												
LIA	−0.003	0.003											
LIB	−0.001	−0.006	−0.003										
LNTZ	0.012	0.006	0.002	−0.004									
NW	**0.015**	−0.001	0.002	−0.005	0.000								
SW	0.009	0.001	−0.001	0.000	0.007	0.004							
ND	**0.011**	0.003	−0.001	−0.002	0.004	0.002	−0.003						
SD	0.004	0.004	0.005	−0.001	**0.007**	**0.010**	0.001	0.000					
WF	0.008	−0.002	0.003	−0.006	0.007	0.000	0.004	−0.001	0.001				
Wex	0.006	−0.001	−0.002	−0.006	−0.002	−0.001	−0.003	0.001	0.005	0.000			
M	0.006	−0.003	0.002	−0.006	0.003	0.002	0.002	−0.002	−0.001	−0.003	0.001		
OX	0.006	0.001	0.003	−0.004	0.005	0.002	−0.004	−0.001	−0.002	−0.002	0.001	0.000	
NNW	**0.014**	**0.010**	**0.014**	0.002	**0.019**	**0.008**	0.002	0.008	0.010	0.001	0.006	**0.009**	0.002
NSE	0.005	0.004	0.008	0.001	**0.023**	0.004	0.003	0.007	**0.016**	0.005	0.009	0.007	0.005
NSW	**0.028**	**0.008**	**0.023**	0.004	**0.016**	**0.012**	0.010	**0.016**	**0.018**	0.008	0.008	**0.013**	0.008
NAB	0.004	0.010	0.011	−0.008	**0.014**	0.009	0.012	0.008	**0.007**	0.003	0.009	0.005	0.008
Pen	−0.001	0.006	0.000	−0.002	0.006	0.008	0.000	0.000	−0.001	−0.001	0.003	−0.001	0.001
7Brt	−0.001	0.003	**0.006**	−0.004	**0.012**	0.005	0.001	0.003	0.000	0.000	0.003	0.000	−0.001
6Jer	0.007	0.006	**0.006**	−0.002	**0.006**	**0.008**	0.001	0.001	0.003	0.001	0.003	0.002	0.000
6Has	0.003	0.005	0.005	−0.003	**0.008**	0.008	−0.002	0.000	−0.002	0.000	0.003	−0.001	−0.001
6Har	0.006	0.004	0.005	−0.005	**0.007**	0.007	0.000	0.001	0.001	0.000	0.003	−0.001	0.000
6Sea	0.004	0.005	0.004	−0.005	0.002	0.007	−0.001	−0.001	−0.001	0.001	0.005	−0.003	−0.001
NNO	**0.016**	0.006	**0.012**	0.000	**0.009**	**0.011**	0.003	0.000	0.002	0.001	**0.007**	−0.002	0.002

	NNW	NSE	NSW	NAB	Pen	7Brt	6Jer	6Has	6Har	6Sea
NSE	0.003									
NSW	0.003	**0.019**								
NAB	0.009	0.009	**0.019**							
Pen2	**0.013**	**0.010**	**0.024**	0.005						
7Brt	0.003	0.002	**0.014**	0.003	0.002					
6Jer	**0.008**	**0.012**	**0.009**	0.009	0.004	0.003				
6Has	0.006	**0.010**	**0.013**	0.003	0.001	0.000	0.001			
6Har	0.005	**0.008**	**0.011**	0.003	0.001	0.002	0.003	0.000		
6Sea	**0.011**	**0.011**	**0.016**	0.003	−0.002	0.002	0.001	−0.002	−0.002	
NNO	**0.010**	**0.012**	**0.014**	0.005	0.002	0.003	0.006	0.000	0.000	−0.002

Values within shaded area are estimated from 11 microsatellite loci while values in clear area are estimated from 8 microsatellite loci. See Table 1 and Supplementary Table 1 for sample codes. F_{ST} values identified as statistically significant following 10,000 permutations are in bold.

performed for each sample or globally across all samples. HWE tests for each locus/sample comparison reported 20 cases of deviations, in all cases due to heterozygote deficits. Locus Cpag-4 exhibited significant heterozygote deficits for 6 (CB, LIA, LNTZ, NW, WF, WEX) out of 13 samples, no other locus exhibited more than 3 deviations from HWE. No sample exhibited significant heterozygote deficits at more than 2 loci except LIA (5) and WEX (3).

Among the Irish Sea samples global tests of differentiation were not significant ($F_{ST} = 0.001$; $P = 0.15$; Exact $P = 0.7$). No locus exhibited any signal of non-neutral evolution, i.e. no significant outliers identified (Supplementary Figure 1). Pairwise F_{ST} values were very low with only 4 out of 78 tests significant (Table 2), while all pairwise exact tests were not significant. Simulation analysis indicated that exact tests based on comparisons between the average sample sizes had a probability of 0.98 of detecting differentiation at a level of $F_{ST} = 0.01$ and a Type I error rate of 0.05. There was no significant IBD effect ($r = 0.005$; Mantel $P = 0.48$) and Bayesian clustering analysis unanimously supported a model of $K = 1$ ($P > 0.99$; ∼0 for all other K values tested).

Genetic diversity across the NE Atlantic

Upon integrated analysis of the Irish Sea samples and the selected samples from McKeown *et al.* (2017) for the 8 loci common to

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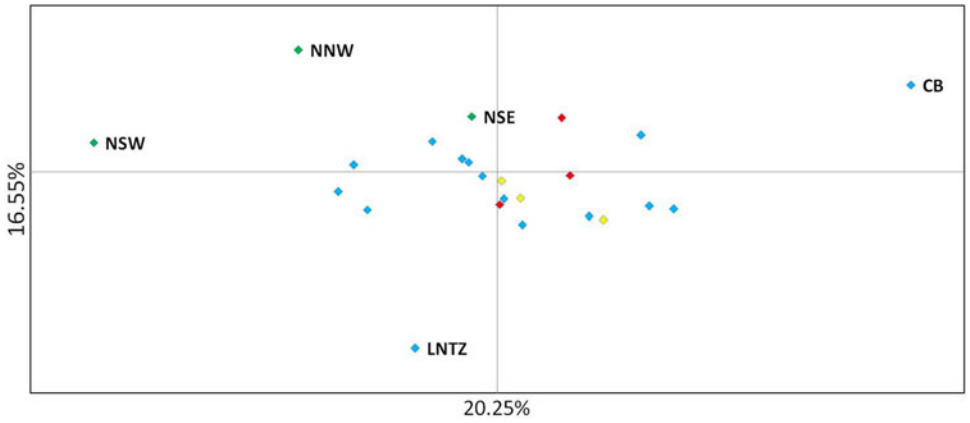

Fig. 2. Principal coordinate analysis of multi-locus pairwise F_{ST} based on 8 microsatellite loci. Irish differentiated samples are labelled as are the Lundy NTZ and Cardigan Bay sample. Remaining samples are colour coded as to location (blue – Irish Sea/Bristol Channel; red – English Channel; yellow – North Sea).

both studies, global F_{ST} remained numerically small but was statistically significant ($F_{ST} = 0.003$; $P < 0.001$). The corresponding global exact test was not significant ($P = 0.7$). All pairwise exact tests were also not significant and Bayesian clustering analysis also failed to detect structuring ($P = 1$ for $K = 1$). Inspection of pairwise F_{ST} values indicated that while the majority of pairwise comparisons within and between regions were not significant certain samples were associated with a large number of significant F_{ST} values. Among these were the three Irish samples from McKeown *et al.* (2017) (west coast: NNW and NSW; south-east coast: NSE) while the Lundy NTZ sample exhibited significant F_{ST} values in comparisons with a number of samples collected from outside the Irish Sea (Table 2 and Figure 2). The differentiation of these samples could be described as patchy in that comparisons between geographically close samples often reported larger F_{ST} than comparisons with more distant samples. There was no significant isolation by distance effect ($r = 0.155$; Mantel $P = 0.15$) and AMOVA revealed more differentiation within regions ($F_{SC} = 0.001$; $P = 0.013$) than between regions ($F_{CT} = 0.0004$; $P = 0.13$) (three regions used in AMOVA: 1 – Irish/Celtic Seas, 2 – English Channel, 3 – North Sea). There was no difference in global estimates of genetic differentiation when samples were partitioned according to sex (e.g. global F_{ST} males/females = 0.001/0.003; two-tailed test $P = 0.325$). All loci conformed to neutral expectations (Supplementary Figure 2) and levels of intrasample genetic variability were comparable across all samples (Supplementary Table 2).

Discussion

The investigation of demographic patterns over multiple spatial scales is vital for the optimization of spatially explicit conservation strategies at both local and regional levels. In this study microsatellite markers were used to investigate population processes within the Irish Sea, including comparisons between the Lundy NTZ and surrounding unprotected areas at 11 loci. Regional patterns were then assessed by integrative analysis of data from McKeown *et al.* (2017) for a subset of 8 loci common to both studies. Consistent with expectations of larval and adult mixing we found no evidence for genetic structuring among samples within the Irish Sea/Bristol Channel, including the Lundy NTZ. On a wider geographic scale the lack of spatial structure was also consistent with high levels of genetic connectivity among the Irish/Celtic Seas, English Channel and North Sea. A number of samples, including the Lundy NTZ exhibited patchy genetic differentiation, that has previously been described for the species and attributed to spatio/temporal recruitment heterogeneity occurring against a background of high gene flow (McKeown *et al.*, 2017). The study also shows that brown crabs retain high levels of genetic variation among samples despite being heavily exploited with no difference in levels of variation for the Lundy NTZ and surrounding 'fished' areas.

Global and pairwise tests of genetic differentiation among Irish Sea samples were mostly non-significant. 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). STRUCTURE analysis, which can distinguish admixed population units failed to find any evidence of population differentiation, though resolution of such analyses may be limited when differentiation among groups is subtle (Latch *et al.*, 2006). Brown crab males and females exhibit markedly different migration patterns with females undertaking long migrations while males are regarded as being largely resident (Edwards, 1979; Bennett and Brown, 1983; Latrouite & Le Foll, 1989; Ungfors *et al.*, 2007). Similar to results from McKeown *et al.* (2017) analysis of sex partitioned samples revealed no significant differentiation among males that could indicate an underlying structure that is being obscured by adult female migration. Genetic analyses of early life history stages (e.g. juvenile recruits) have in some taxa revealed structuring/cohesion not detectable in older life history stages due to ontogenetic mixing (e.g. Christie *et al.*, 2010). A number of the Irish Sea samples analysed here were comprised exclusively of juveniles which must be considered unlikely to have undertaken any extensive migrations. However, these samples also exhibited no signals of genetic differentiation. Such genetic homogeneity is readily consistent with results from other studies. Biophysical modelling of larval dispersal for a number of species has predicted that the hydrography of the region would facilitate larval dispersal between Irish and British coasts (Coscia *et al.*, 2013; Gormley *et al.*, 2015). In a study employing near identical sample collection sites to this one, Watson *et al.* (2016) found no genetic structure across the Irish and Celtic Sea in the European lobster (*Homarus gammarus*), a species for which dispersal is restricted to the larval stage unlike crab where postlarval dispersal is reported. Watson *et al.* (2016) also reported no differences between the Lundy NTZ and other Irish Sea/Bristol Channel samples. The lack of population genetic structure indicates high levels of gene flow within and between the Irish Sea and Bristol Channel.

At the regional level, the majority of pairwise comparisons between the Irish Sea/Bristol Channel and samples from the English Channel and North Sea were largely non-significant. Corresponding AMOVA among-region indices of variation were not significant and there was no isolation by distance effect. A lack

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of genetic differentiation between Irish Sea and surrounding waters has been reported for a number of marine species such as plaice *Pleuronectes platessa* (Was *et al.*, 2010), flounder *Platichthys flesus* (Hemmer-Hansen *et al.*, 2007) and gilthead sea bream *Sparus aurata* (Coscia *et al.*, 2012). Overall, the results here, along with the brown crab population genetic studies by McKeown *et al.* (2017) and Ungfors *et al.* (2009) support high gene flow among the Irish Sea, Celtic Sea, English Channel and North Sea (including the Kattegat and Skagerrak). Weak, or lack of, genetic structuring along the European Atlantic coast has also been reported for a number of invertebrate species with high larval dispersal potential, such as the green crab *Carcinus maenas* (Domingues *et al.*, 2010), sea urchin *Paracentrotus lividus* (Duran *et al.*, 2004), European lobster *H. gammarus* (Triantafyllidis *et al.*, 2005), spider crab *Maja brachydactyla* (Sotelo *et al.*, 2008) and velvet swimming crab *Necora puber* (Sotelo *et al.*, 2009). Some recent population genetic studies of lobster (Ellis *et al.*, 2017) and great scallop (*Pecten maximus*) (Morvezen *et al.*, 2016) have revealed signs of restricted gene flow between Scandinavian and other Atlantic populations linked to oceanographic retention of larvae. However, in the case of brown crab, even if larval dispersal is restricted gene flow may be facilitated by postlarval dispersal. Such postlarval dispersal has been implicated as maintaining recurrent gene flow across the Flamborough frontal system in the North Sea which has been highlighted as a predicted physical barrier to dispersal of brown crab larvae between areas north and south of the front (Eaton *et al.*, 2003).

In the study by McKeown *et al.* (2017) spanning the NE Atlantic, despite geographically extensive gene flow, specific samples exhibited significant differentiation from a number of other samples but this differentiation was spatially and temporally 'patchy' in the sense that differentiation could be greater between temporal replicates from a given site and/or a geographically nearby site than between more distant samples. This fitted with the commonly reported pattern of chaotic genetic patchiness (CGP) (Johnson & Black, 1982) and, as is often the case, was interpreted as being due to reproductive skews (sweepstakes recruitment) and/or larval cohesion generating genetic differences despite gene flow. The three Irish samples common to both studies (NNW, NSW, NSE) exhibited CGP in both the original study (McKeown *et al.*, 2017) and in comparisons with the new Irish Sea/Bristol Channel data reported here. Within the combined 8 loci data set the Lundy NTZ yielded a number of significant test results in comparisons between samples from the English Channel and North Sea, however this differentiation could also be described as patchy as the Lundy NTZ was not differentiated from most Irish Sea samples that were themselves largely homogeneous with the Channel and North Sea samples. McKeown *et al.* (2017) suggested larval retention along complex coastlines as a driver of the CGP and as the Lundy NTZ is located at the mouth of the Bristol Channel similar seascape drivers could be involved. Although no patchiness was observed for the Lundy control samples a significant heterozygote deficit was observed for Lundy control sample A. Such deficits among samples of mixed cohorts may stem from recruitment skews within, and thus differentiation among, cohorts (Harvey *et al.*, 2016). Watson *et al.* (2016) reported genetic signals compatible with sweepstakes recruitment among European lobster samples in the Lundy NTZ and suggested that this may be linked to increased variances in reproductive success due to the presence of larger lobsters compared with surrounding fished areas. McKeown *et al.* (2017) similarly suggested that the greater fecundity of crabs in the English Channel and Celtic Sea compared with the North Sea, where no patchiness was detected, may increase the chance of sweepstakes recruitment in those regions. In keeping with this theme Palero *et al.* (2011) posit 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. While a confirmation of the processes driving the patchy genetic structure will require cohort specific analysis (Christie *et al.*, 2010) the signals of altered recruitment dynamics for two crustacean species (brown crab and lobster) within the Lundy NTZ warrant further investigation.

McKeown & Shaw (2008*b*) suggested that the combination of female genetic monogamy placing a limit on the number of males that can successfully breed, alongside the selective harvesting of females may contribute to fishery driven genetic erosion. However, levels of genetic variability were consistently high across all samples and similar to values reported for other NE Atlantic decapods (e.g. Sotelo *et al.*, 2008, 2009; Domingues *et al.*, 2010). In a study of two MPAs in the western Mediterranean by Perez-Ruzafa *et al.* (2006) higher values of total and standardized allelic richness were observed in protected populations of *Diplodus sargus sargus* than in unprotected ones. Here similar levels of variability were reported for the Lundy NTZ and other samples. Calo *et al.* (2016) also reported similar levels of genetic diversity for protected and unprotected areas in the saddled sea bream (*Oblada melanura*). While we do not have samples from before the commencement of commercial harvesting or during the initial stages of exploitation when loss of diversity may be most pronounced (Ryman *et al.*, 1995) and thus cannot rule out the possibility that some genetic variation has been lost due to fishing, the results indicate that high levels of genetic variation are maintained within the species which is fundamental for maintaining sustainable yields and adaptability of populations (Kenchington *et al.*, 2003). As for many other marine species that maintain high levels of genetic variation despite high levels of exploitation, genetic loss may be being buffered by factors such as gene flow, overlapping generations and high mutation rates (Kennington *et al.*, 2013).

In conclusion, our analyses support the view that brown crabs within the Irish Sea and surrounding sampled areas are derived from a single genetically panmictic population and suggest that if current spawning stock sizes and management practises are maintained genetic drift is not strong enough to reduce neutral genetic diversity. The high connectivity throughout the studied area has implications for the distribution of future MPAs (Larson & Julian, 1999) and emphasizes the need for international cooperation in the management of the resource. This is a timely consideration in light of the potential for mismatch between biological and geopolitical stock boundaries in the Irish Sea after Brexit. A key aspect of such management should be the application of genomic approaches which may detect population isolation and adaptation patterns beyond the resolution of this study (King *et al.*, 2017) and that can be used to disentangle the drivers of genetic patchiness in this species.

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