

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

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*Understanding demographic processes over multiple spatial scales is vital for the optimisation 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.*

Keywords: population, fishery, crustacean, Marine Protected Areas, sustainability, microsatellites.

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 geographical 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 optimisation 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 (Hunter et al. 2013; Ungfors et al. 2007). As the larvae are poor swimmers likely to passively drift while entrained in currents, it has been suggested that contranatal 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 geographical areas. Ungfors et al., (2009), using microsatellite markers, reported no significant genetic differentiation among samples spanning 1300km 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 United Kingdom’s (UK) 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 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; Goff & Moon 1993). Individuals were then genotyped at 11 species-specific microsatellite loci developed by (McKeown & Shaw 2008a) 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 μ l, containing 7.5 μ l of Multiplex Kit Buffer and 1 μ l of genomic DNA. Primer volumes varied: 0.35 μ l Cpag-4C1; 0.3 μ l Cpag-1B9, Cpag-3A2, Cpag-3D7, Cpag-4 and Cpag-5D8; 0.25 μ l Cpag-38; 0.2 μ l Cpag-1C8, Cpag-6C4B and Cpag-15; and 0.1 μ l Cpag-2A5B. 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 55 °C and 45 seconds at 72 °C, and a final extension step at 72 °C for 45 minutes. 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 characterised 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 visualised using principal coordinate analysis in GENALEX. Mantel tests, implemented in GENALEX were used to test for correlation between pairwise F_{ST} and geographical distances between sample sites (i.e. isolation by distance (IBD)). Geographical 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 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. Randomisation 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 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 six (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 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 ($F_{CT} = 0.0004$; $P = 0.13$) (3 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 2 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 optimisation 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 geographical 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. 2007). 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 & 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 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 homogenous 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 to surrounding fished areas. McKeown et al., (2017) similarly suggested that the greater fecundity of crabs in the English Channel and Celtic Sea compared to the North Sea, where no patchiness was detected, may increase the chance of sweepstake 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 et al., (2008) 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 standardised 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., (2014) 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 emphasises 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.

ACKNOWLEDGEMENTS

The Irish Sea data collection was performed as part of SUSFISH, a 3 year project funded by the European Union Regional Development fund (ERDF) under the Ireland Wales Programme 2007-2013 (interreg4A, Project No. 042) while the concatenated data analysis was performed as part of the EU BLUEFISH project.

LITERATURE CITED

- Almany G.R., et al.** (2009) Connectivity, biodiversity conservation and the design of marine reserve networks for coral reefs. *Coral Reefs*, **28**(2), 339-351.
- Antao T., Lopes A., Lopes R.J., Beja-Pereira A. and Luikart G.** (2008) LOSITAN: A workbench to detect molecular adaptation based on a F(st)-outlier method. *Bmc Bioinformatics*, **9**: 323.
- Babbucci M., et al.** (2010) Population structure, demographic history, and selective processes: Contrasting evidences from mitochondrial and nuclear markers in the European spiny lobster *Palinurus elephas* (Fabricius, 1787). *Molecular Phylogenetics and Evolution*, **56**(3), 1040-1050.
- Bennett D.B.** (1995) Factors in the life history of the edible crab (*Cancer pagurus* L.) that influence modelling and management. *ICES Marine Science Symposia*, **199**(0), 89-98.
- Bennett D.B. and Brown CG** (1983) Crab (*Cancer pagurus*) migration in the English Channel. *Journal of the marine Biological Association of the United Kingdom*, **63**: 371-398.
- Calo A., Munoz I., Perez-Ruzafa A., Vergara-Chen C. and Garcia-Charton J.A.** (2016) Spatial genetic structure in the saddled sea bream (*Oblada melanura* Linnaeus, 1758) suggests multi-scaled patterns of connectivity between protected and unprotected areas in the Western Mediterranean Sea. *Fisheries Research*, **176**, 30-38.
- Christie M.R., et al.** (2010) Larval Connectivity in an Effective Network of Marine Protected Areas. *Plos One*, **5**(12).
- Coscia I., Robins P.E., Porter J.S., Malham S.K. and Ironside J.E.** (2013) Modelled larval dispersal and measured gene flow: seascape genetics of the common cockle *Cerastoderma edule* in the southern Irish Sea. *Conservation Genetics*, **14**(2), 451-466.
- Coscia I., Vogiatzi E., Kotoulas G., Tsigenopoulos C.S. and Mariani S.** (2012) Exploring neutral

and adaptive processes in expanding populations of gilthead sea bream, *Sparus aurata* L., in the North-East Atlantic. *Heredity*, **108**(5), 537-546.

- Domingues C.P., Creer S., Taylor M.I., Queiroga H. and Carvalho G.R.** (2010) Genetic structure of *Carcinus maenas* within its native range: larval dispersal and oceanographic variability. *Marine Ecology Progress Series*, **410**, 111-123.
- Duran S., Palacin C., Becerro M.A., Turon X. and Giribet G.** (2004) Genetic diversity and population structure of the commercially harvested sea urchin *Paracentrotus lividus* (Echinodermata, Echinoidea). *Molecular Ecology*, **13**(11), 3317-3328.
- Eaton D.R., Brown J., Addison J.T., Milligan S.P. and Fernand L.J.** (2003) Edible crab (*Cancer pagurus*) larvae surveys off the east coast of England: implications for stock structure. *Fisheries Research*, **65**(1-3), 191-199.
- Edwards E.** (1979) The edible crab and its fishery in British waters. Fishing News Books, Surrey, England.
- Ellis C.D., Hodgson D.J., Daniels C.L., Collins M. and Griffiths A.G.F.** (2017) Population genetic structure in European lobsters: implications for connectivity, diversity and hatchery stocking. *Marine Ecology Progress Series*, **563**, 123-137.
- Excoffier L. and Lischer H.E.L.** (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**(3), 564-567.
- Excoffier L., Smouse P.E. and Quattro J.M.** (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes – application to human mitochondrial DNA restriction data. *Genetics*, **131**(2), 479-491.
- Gormley K., et al.** (2015) Connectivity and Dispersal Patterns of Protected Biogenic Reefs: Implications for the Conservation of *Modiolus modiolus* (L.) in the Irish Sea. *Plos One*, **10**(12).
- Goudet J.** (1995) FSTAT (Version 1.2): A computer program to calculate F-statistics. *Journal of Heredity*, **86**(6), 485-486.
- Harvey B.P., et al.** (2016) Individual and population-level responses to ocean acidification. *Scientific Reports*, **6**, article 20194.
- Hastings A. and Botsford L.W.** (2006) Persistence of spatial populations depends on returning home. *Proceedings of the National Academy of Sciences of the United States of America*, **103**(15), 6067-6072.
- Hedgecock D.** (1994) Does variance in reproductive success limit effective population sizes of marine organisms. *Genetics and Evolution of Aquatic Organisms*, 122-134.
- Hemmer-Hansen J., Nielsen E.E., Gronkjaer P. and Loeschcke V.** (2007) Evolutionary mechanisms shaping the genetic population structure of marine fishes; lessons from the European flounder (*Platichthys flesus* L.). *Molecular Ecology*, **16**(15), 3104-3118.
- Hubisz M.J., Falush D., Stephens M. and Pritchard J.K.** (2009) Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, **9**(5), 1322-1332.
- Hunter E., Eaton D., Stewart C., Lawler A. and Smith M.T.** (2013) Edible Crabs "Go West": Migrations and Incubation Cycle of *Cancer pagurus* Revealed by Electronic Tags. *Plos One*, **8**(5).
- Johnson M.S., & Black R.** (1982) Chaotic genetic patchiness in an intertidal limpet *Siphonaria* sp. *Marine Biology*, **70**, 157-164.
- Jorde P.E., et al.** (2015) Genetically distinct populations of northern shrimp, *Pandalus borealis*, in the North Atlantic: adaptation to different temperatures as an isolation factor. *Molecular Ecology*, **24**(8), 1742-1757.
- Kenchington E., Heino M. and Nielsen E.E.** (2003) Managing marine genetic diversity: time for action? *ICES Journal of Marine Science*, **60**(6), 1172-1176.

- Kennington W.J., Cadée S.A., Berry O., Groth D.M., Johnson M.S. and Melville-Smith R.** (2013) Maintenance of genetic variation and panmixia in the commercially exploited western rock lobster (*Panulirus cygnus*). *Conservation Genetics*, **14**(1), 115-124.
- King N.G., McKeown N.J. Smale, D.A. and Moore P.J.** (2017) The importance of phenotypic plasticity and local adaptation in driving intraspecific variability in thermal niches of marine macrophytes. *Ecography*, **40**, 1-14.
- Kuparinen A., Keith D.M. and Hutchings J.A.** (2014) Increased environmentally driven recruitment variability decreases resilience to fishing and increases uncertainty of recovery. *ICES Journal of Marine Science*, **71**(6), 1507-1514.
- Larson R.J. and Julian R.M.** (1999) Spatial and temporal genetic patchiness in marine populations and their implications for fisheries management. *California Cooperative Oceanic Fisheries Investigations Reports*, **40**, 94-99.
- Latrouite D. and Le Foll D.** (1989) Migrations of the edible crab *Cancer pagurus* and the spider crab *maja squinado*. *Oceanis*, **15**(2), 133-142.
- McKeown N.J., Hauser L. and Shaw P.W.** (2017) Microsatellite genotyping of brown crab *Cancer pagurus* reveals fine scale selection and 'non-chaotic' genetic patchiness within a high gene flow system. *Marine Ecology Progress Series*, **566**, 91-103.
- McKeown N.J. and Shaw P.W.** (2008a) Polymorphic nuclear microsatellite loci for studies of brown crab, *Cancer pagurus* L. *Molecular Ecology Resources*, **8**(3), 653-655.
- McKeown N.J. and Shaw P.W.** (2008b) Single paternity within broods of the brown crab *Cancer pagurus*: a highly fecund species with long-term sperm storage. *Marine Ecology Progress Series*, **368**, 209-215.
- Morvezen R., et al.** (2016) Genetic structure of a commercially exploited bivalve, the great scallop *Pecten maximus*, along the European coasts. *Conservation Genetics*, **17**(1), 57-67.
- Nei M.** (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**(3), 583-590.
- Nielsen E.E., Nielsen P.H., Meldrup D. and Hansen M.M.** (2004) Genetic population structure of turbot (*Scophthalmus maximus* L.) supports the presence of multiple hybrid zones for marine fishes in the transition zone between the Baltic Sea and the North Sea. *Molecular Ecology*, **13**(3), 585-595.
- Palero F., Abello P., Macpherson E., Beaumont M. and Pascual M.** (2011) Effect of oceanographic barriers and overfishing on the population genetic structure of the European spiny lobster (*Palinurus elephas*). *Biological Journal of the Linnean Society*, **104**(2), 407-418.
- Palumbi S.R.** (2003) Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications*, **13**(1), S146-S158.
- Pampoulie C., et al.** (2011) A pilot genetic study reveals the absence of spatial genetic structure in Norway lobster (*Nephrops norvegicus*) on fishing grounds in Icelandic waters. *ICES Journal of Marine Science*, **68**(1), 20-25.
- Pawson M.G.** (1995) Biogeographical identification of English Channel fish and shellfish stocks. *Fisherie research technical report*, MAFF Lowestoft (660: 72pp.)
- Peakall R. and Smouse P.E.** (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**(1), 288-295.
- Perez-Ruzafa A., Gonzalez-Wanguemert M., Lenfant P., Marcos C. and Garcia-Charton J.A.** (2006) Effects of fishing protection on the genetic structure of fish populations. *Biological Conservation*, **129**(2), 244-255.
- Pritchard J.K., Stephens M. and Donnelly P.** (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**(2), 945-959.
- Raymond M. and Rousset F.** (1995) An exact test for population differentiation. *Evolution*, **49**(6), 1280-1283.

- Reiss H., Hoarau G., Dickey-Collas M. and Wolff W.J.** (2009) Genetic population structure of marine fish: mismatch between biological and fisheries management units. *Fish and Fisheries*, **10**(4), 361-395.
- Ryman N. and Palm S.** (2006) POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. *Molecular Ecology Notes*, **6**(3), 600-602.
- Ryman N., Utter F. and Laikre L.** (1995) Protection of intraspecific biodiversity of exploited fishes. *Reviews in Fish Biology and Fisheries*, **5**(4), 417-446.
- Sotelo G., Moran P., Fernandez L. and Posada D.** (2008) Genetic variation of the spiny spider crab *Maja brachydactyla* in the northeastern Atlantic. *Marine Ecology Progress Series*, **362**, 211-223.
- Sotelo G., Posada D. and Moran P.** (2009) Low-mitochondrial diversity and lack of structure in the velvet swimming crab *Necora puber* along the Galician coast. *Marine Biology*, **156**(5), 1039-1048.
- Triantafyllidis A., et al.** (2005) Mitochondrial DNA variation in the European lobster (*Homarus gammarus*) throughout the range. *Marine Biology*, **146**(2), 223-235.
- Ungfors A., Hallback H. and Nilsson P.G.** (2007) Movement of adult edible crab (*Cancer pagurus* L.) at the Swedish West Coast by mark-recapture and acoustic tracking. *Fisheries Research*, **84**(3), 345-357.
- Ungfors A., McKeown N.J., Shaw P.W. and Andre C.** (2009) Lack of spatial genetic variation in the edible crab (*Cancer pagurus*) in the Kattegat-Skagerrak area. *ICES Journal of Marine Science*, **66**(3), 462-469.
- Waples R.S., Punt A.E. and Cope J.M.** (2008) Integrating genetic data into management of marine resources: how can we do it better? *Fish and Fisheries*, **9**(4), 423-449.
- Was A., Gosling E. and Hoarau G.** (2010) Microsatellite analysis of plaice (*Pleuronectes platessa* L.) in the NE Atlantic: weak genetic structuring in a milieu of high gene flow. *Marine Biology*, **157**(3), 447-462.
- Watson H.V., McKeown N.J., Coscia I., Wootton E. and Ironside J.E.** (2016) 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. *Fisheries Research*, **183**, 287-293.
- Weir B.S. and Cockerham C.C.** (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**(6), 1358-1370.
- Weiss M., Thatje S., Heilmayer O., Anger K., Brey T. and Keller M.** (2009) Influence of temperature on the larval development of the edible crab, *Cancer pagurus*. *Journal of the Marine Biological Association of the United Kingdom*, **89**(4), 753-759.