Vol. 566: 91–103, 2017
doi: 10.3354/meps12044

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published February 27

Microsatellite genotyping of brown crab *Cancer pagurus* reveals fine scale selection and 'non-chaotic' genetic patchiness within a high gene flow system

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ABSTRACT: Brown crab *Cancer pagurus* supports one of the most important fisheries in Europe; however, spatial patterns of connectivity and adaptation are largely unknown and difficult to identify due to the species' life history, which entails distinct dispersal characteristics during larval and adult life stages. To address this limitation, spatial–temporal genetic structure using 8 microsatellite loci was assessed across the majority of the species' NE Atlantic distribution. Neutral genetic structuring revealed a background of high gene flow throughout the region, with a superimposed pattern of chaotic genetic patchiness (CGP) linked to stochastic recruitment variability. The CGP was geographically patterned, being prevalent among English Channel samples but absent among North Sea samples, suggesting specific biological (e.g. reproductive ecology) and environmental (seascape) drivers. Such recruitment variability may compromise stock resilience and must be considered within spatial management strategies. Another prominent feature was pronounced differentiation at a single locus for males sampled within a single fjord (Gulmarsfjord) from all other samples, exhibiting the effects of divergent selection. Gulmarsfjord females were genetically similar to all other 'non-fjord' samples, and exhibited a comparative level of differentiation at the outlier locus from the Gulmarsfjord males. Due to known dispersal differences between the sexes, the pattern within Gulmarsfjord can be explained by the intermingling of allochthonous females with resident, locally adapted males and demonstrates the occurrence of fine-scale local adaptation in this species. Collectively, the study highlights how considerable intraspecific eco-evolutionary diversification can occur despite high levels of dispersal/gene flow.

KEY WORDS: Adaptation · Gene flow · Dispersal · Sweepstakes recruitment · Conservation · Sustainability

INTRODUCTION

Genetic studies have yielded many insights into marine intraspecific biodiversity, with important findings including the detection of significant genetic population structuring (Shaw et al. 1999, Knutsen et al. 2011, McKeown et al. 2015) and adaptation (Hemmer-Hansen et al. 2007a, Poulsen et al. 2011, Therkildsen et al. 2013a) in systems where high gene flow would be expected to prevent such differentiation (Palumbi 1994, Waples 1998). Population genetic structure and adaptation, as components of intraspecific biocomplexity, are thought to be significant factors underpinning species/population sustainability and evolutionary potential (Iles & Sinclair 1982, Ryman et al. 1995, Ruzzante et al. 2006, Therkildsen et al. 2013b). The ongoing depletion of marine populations through fishing, and demographic changes

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associated with predicted future climate change, are adding to the impetus to resolve (1) spatial/temporal patterns of neutral and adaptive genetic structure (Reiss et al. 2009), (2) historical and contemporary drivers of structuring (Hemmer-Hansen et al. 2007b), and (3) the significance of such diversity on ecological and evolutionary time scales (Stepien et al. 2009).

While there has been considerable research on fishes with mobile larval and non-larval stages, 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.), which occurs continuously in shallow shelf waters of the NE Atlantic from the Lofoten Islands (Norway) to Morocco (Bennett 1995) and supports one of the most important commercial European fisheries, represents an interesting candidate for investigation as both larval and adult stages have the potential for substantial dispersal. 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 (nmi) have been reported with some crabs achieving a mean speed of 1.07 to 1.62 nmi d^{-1} (Pawson 1995). The pelagic larval stage lasts for approximately 3 mo (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 occur consistently against prevailing currents (Ungfors et al. 2007, Hunter et al. 2013). As the larvae are poor swimmers and likely passively drift while entrained in currents, it has been suggested that contranatent female migrations are spawning behaviours aimed at facilitating return to areas of maternal origin. Even in the absence of additional extrinsic factors, the seemingly counteractive dispersal of females and larvae is expected to limit 'lifetime dispersal' and may thus influence spatial patterns of recruitment and structuring of reproductive populations. Tagging, fishery landings data and sex-specific growth rates variously suggest some demographic independence between the areas of brown crab abundance in the Celtic Sea, English Channel, North Sea and Bay of Biscay (Pawson 1995). Within the North Sea, the seasonal jet-like circulation associated with the Flamborough front is predicted to prevent exchange of larvae between areas north and south of the front during spawning time (Eaton et al. 2003). In the English Channel, larval surveys have reported distinct western and eastern centres of larval abundance separated by a central area of low or no larval occurrence, and hydrodynamic modelling has indicated insufficient larval transport rates to connect these spawning areas (D. Eaton unpubl. data).

To date, population genetic structure of brown crab has been studied only in Scandinavian waters, where Ungfors et al. (2009) reported no significant genetic differentiation among samples spanning 1300 km of waterway distance within the Norwegian Sea, Skagerrak and Kattegat. However, genetic structuring may vary throughout a species' range, and failure to identify local populations may lead to local overfishing and ultimately, severe declines. While females are highly fecund (0.5 to 2.9 million eggs per brood; Edwards 1979, Ungfors 2007), paternity analysis suggests single paternity of broods (McKeown & Shaw 2008b). Such a reproductive ecology, alongside the selective harvesting of females (Bennett 1995), which are currently regarded as overexploited, may enhance the susceptibility of brown crab to genetic erosion (McKeown & Shaw 2008b).

The objective of the present study was to test the general hypothesis of genetic panmixia in brown crab throughout a considerable portion of the species' range, with a specific focus on the English Channel and North Sea. Some genetic studies of crustaceans have reported macro-geographical homogeneity with structuring apparent only at regional scales (e.g. Domingues et al. 2010), while other studies have reported fine-scale spatial and/or temporal genetic structuring (Selkoe et al. 2010). To encapsulate such potential complexity, broad- and fine-scale spatial–temporal patterns were assessed. Furthermore, comparative analyses of males and females were performed to identify differences that may be associated with sex-specific ontogenetic movements. The sampling strategy also encompassed distinct seascape features (e.g. samples collected within semi-enclosed water bodies such as bays and fjords) to examine the effect of local hydrodynamic environments. This sampling design permitted interpretation of the mechanistic underpinnings and eco-evolutionary significance of complex patterns of genetic diversity which included evidence of broad-scale genetic connectivity, fine-scale adaptive divergence of a fjord sample, and regional variation in genetic patchiness.

MATERIALS AND METHODS

Sample collection and molecular analyses

Spatial/temporal sampling of adults throughout the NE Atlantic was performed using both research (Centre for Environment, Fisheries and Aquaculture Science; CEFAS) and commercial vessels (Fig. 1; see also Table 1 for sample information). For each sample, crabs were captured using multiple baited pots within a localised area (maximum distance among pots ~200 m) over a single day, with tissue biopsies preserved in ethanol. Although adult crabs cannot be reliably aged, samples were considered to consist of multiple age cohorts. For samples collected on-board CEFAS vessels, the majority of individuals were identified as male or female, which permitted downstream separation in statistical analysis.

Total DNA was extracted using a standard CTAB-chloroform/isoamylalcohol method (Winnepenninckx et al. 1993). All individuals were typed at 8 microsatellite loci (*Cpag15*, *Cpag1b9*, *Cpag2a5-2*, *Cpag3a2*, *Cpag3d7*, *Cpag4*, *Capg5d8*, *Cpag6c4b*) following McKeown & Shaw (2008a).

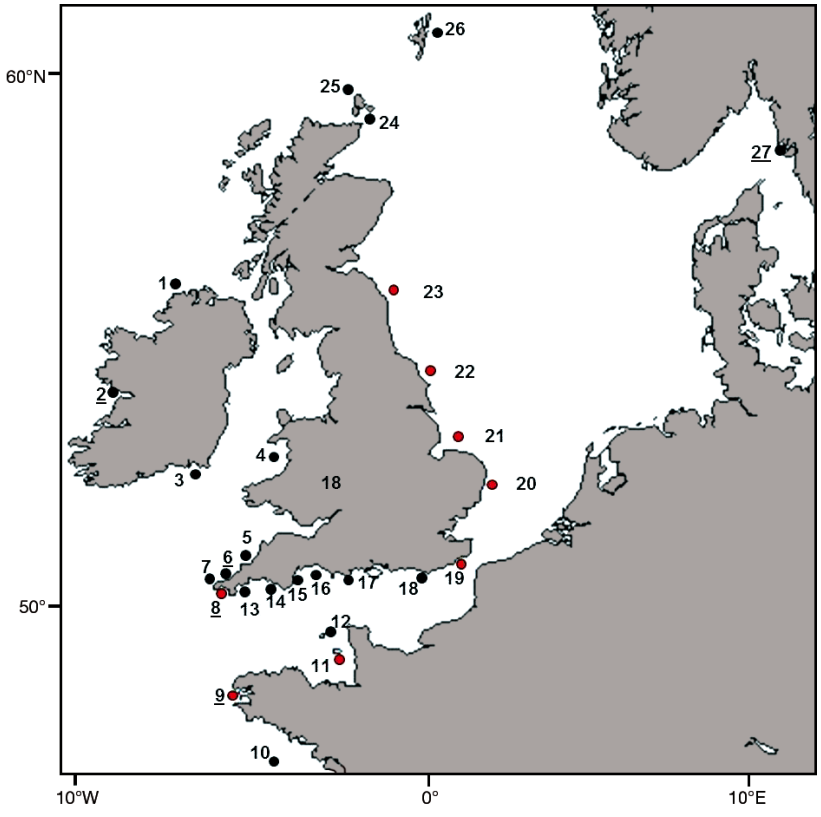

Fig. 1. Brown crab *Cancer pagurus* sample sites (see Table 1 for site details). Red dots: sites with temporal replicates; underlined numbers: sites within semi-enclosed bays and fjords

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). 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. Permutation of genotypes among all samples (999 times) was used to calculate the upper and lower 95% confidence intervals for the expected range of r_{qg} under a panmictic model.

Genetic differentiation was quantified by global and pairwise F_{ST} values, with associated significance evaluated by 10 000 permutations (Goudet et al. 1996), using FSTAT. 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. To help visualise F_{ST} results, principal coordinates analysis (PCoA) was performed on pairwise matrices. Mantel tests, as implemented in the IBDWS software (Jensen et al. 2005) were used to test for correlation between pairwise linearised F_{ST} ($F_{ST}/[1 - F_{ST}]$) (Rousset 1997) and shortest sea distances between sample sites (i.e. isolation by distance; IBD). IBD tests were based on 10 000 randomisations and performed on combinations of untransformed and log-transformed genetic and geographical distances for various pooled and partitioned arrangements of temporal, male and female samples. Differentiation between samples was tested with global and pairwise exact *G*-tests 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 the exact *G*-tests. Genetic structuring was also investigated using the Bayesian clustering method in STRUCTURE (Pritchard et al. 2000), both with and without prior population information and with multiple parameter sets (i.e. with and without admixture, and with and without correlated allele frequencies). 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.

The assumption of selective neutrality of the microsatellite loci was assessed using the FDIST outlier identification test (Beaumont & Nichols 1996) implemented in LOSITAN (Antao et al. 2008) performed (1) globally (i.e. across groups of samples) and (2) between pairs of samples. Simulations were run for 10 000 replications, and 95% confidence intervals were estimated using the options for neutral and forced mean F_{ST}. No differences were detected between analyses assuming infinite allele model (IAM) and stepwise mutation models, so only IAM results are presented.

RESULTS

Intrasample genetic variability and data power

A total of 2777 individuals were assayed (mean sample size: 81.7), with an average of 17.4 alleles detected per locus (range: 5 to 34). Loci *Cpag4* and *Cpag5d8* had considerably more alleles (n = 34 in both cases) than the other loci, with the next highest allele number reported for *Cpag1b9* (19 alleles). Each locus was polymorphic in all samples with very similar levels of variation across all samples. No significant linkage disequilibrium between loci was detected, either across all samples (data pooled) or in any single sample. Single locus tests for conformity to HWE expectations for each of the initial 34 samples (Table 1) revealed the largest number of significant deviations (at critical p value = 0.05) for *Cpag4* and *Cpag3A2* which exhibited 19 and 12 significant test results, respectively. No other locus exhibited more than 5 deviations out of 34 tests (at critical p = 0.05). Multi-locus tests of HWE and associated F_{IS} values were non-significant in most samples (Table 1). All single- and multi-locus deviations from HWE were due to heterozygote deficits. Application of MICRO-CHECKER (van Oosterhout et al. 2006) algorithms to adjust for potential null alleles in cases of single locus heterozygote deficits resulted in no change to the magnitude and pattern of genetic differentiation revealed in subsequent tests, so results for unedited data are reported. Mean intra-sample relatedness conformed to predictions of a panmictic model for all but 2 samples (Table 1).

POWSIM analysis indicated both considerable statistical power for *G*-tests to detect population structure and low Type I error rates for various sample size permutations relative to this study (Table 2).

Detection of divergent selection effects

Significant differentiation was detected between males and females collected in the Gulmarsfjord (F_{ST} = 0.027, p < 0.0001; exact *G*-test p < 0.0001). This differentiation was driven by a single locus (*Cpag6c4b*), which yielded a pairwise F_{ST} of 0.160 (p = 0.0001; exact *G*-test p < 0.0001). Differentiation between the sexes was not significant upon exclusion of this locus (F_{ST} = 0.0048, p = 0.20; exact *G*-test p = 0.06). Genotype proportions at *Cpag6c4b* conformed to HWE among both Gulmarsfjord males (p = 0.5) and females (p = 0.8), with both groups exhibiting nearly identical levels of variability at this locus as well as at other loci. The locus-specific differentiation between the sexes was affected by a clear shift in respective allele frequency distributions (Fig. 2). The simulation-based test for signals of selection within male and female samples identified *Cpag6c4b* as a positive outlier (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m566p091_supp.pdf; simulated neutral F_{ST} smaller than observed F_{ST} for *Cpag6C4b*, p = 0.9979), likely to be influenced by divergent selection.

Comparison of the Gulmarsfjord samples with all other samples revealed (1) the distinctiveness of the Gulmarsfjord males, and (2) the relative similarity of the Gulmarsfjord females to all other samples (Table 3). The pronounced differentiation of the Gulmarsfjord males from all other samples was also driven by locus *Cpag6c4b* (mean ± SD pairwise F_{ST} for *Cpag6c4b*: 0.169 ± 0.013); mean pairwise F_{ST} excluding *Cpag6c4b*: 0.003 ± 0.003). Outlier identification tests identified *Cpag6c4b* as a positive outlier in all pairwise and global tests that included the Gulmarsfjord males (Fig. S2 in the Supplement). *Cpag6c4b* allele frequency distributions among Gulmarsfjord females were similar to other samples (Fig. 2), and pairwise comparisons including this sample yielded a mean pairwise F_{ST} for *Cpag6c4b* of

Table 1. Brown crab *Cancer pagurus* sample information, including geographical region (used in AMOVA), date of collection and sample composition (i.e. numbers of males/females where identified at time of sampling). Multi-locus genetic variability measures: N_A: allele number; A_R: allele richness; H_o: observed heterozygosity; H_e: expected heterozygosity; F_{IS}: standardised genetic variance within samples; r_{qg}: within-sample relatedness

Region/ sample site	Sample code	Sample no.	Date collected	Sample composition				Mean N_A locus^{-1}	Mean A_R	H_o	H_e	F_{IS}	r_{qg}
				Male	Female	Gender unknown	Total						
West of Ireland													
Northwest Ireland	NWIre	1	Jul-07			30	30	8.00	7.867	0.601	0.6571	0.086[b]	−0.013
Galway Bay	GalBay	2	Jul-07			46	46	8.63	7.567	0.589	0.6456	0.089[b]	0.029
Irish/Celtic Sea													
Southeast Ireland	SEIre	3	Jul-07			31	31	8.38	8.176	0.624	0.646	0.034	0.011
Aberystwyth	Aber	4	Aug-00	8	61[a]		69	10.00	8.221	0.622	0.630	0.014	0.058[c]
Newquay	Newq	5	Jun-06	43[a]	51[a]		94	10.88	8.196	0.658	0.645	−0.02	0.010
St. Ives Bay	SI Bay	6	Sep-07			55	77	11.13	8.242	0.676	0.649	−0.042	0.008
Pendeen	Pen	7	Jun-06	51[a]	51[a]		102	10.75	7.790	0.598	0.652	0.083[b]	0.004
English Channel													
Newlyn Bay	NewBay	8	Sep-00	1	83[a]		84	11.13	8.357	0.689	0.673	−0.024	−0.019
Newlyn Bay	NewBay	8	Oct-07			81	81	10.50	7.983	0.638	0.664	0.041[b]	0.001
Brittany Bay	Brit Bay	9	Sep-00			58	58	9.00	7.696	0.651	0.660	0.014	0.009
Brittany Bay	Brit Bay	9	Jul-06		102[a]		102	11.38	8.366	0.621	0.646	0.042[b]	0.026
Brittany-Offshore	Brit offshore	10	Oct-06	56[a]	58[a]		114	11.50	8.221	0.626	0.660	0.053[b]	−0.003
Jersey	Jer	11	Sep-00	40[a]	32[a]		72	10.13	7.918	0.662	0.653	−0.014	0.020
Jersey	Jer	11	Sep-07			84	84	10.88	8.230	0.653	0.642	−0.018	0.037[c]
Guernsey	Guer	12	Sep-07			80	80	10.50	8.164	0.659	0.659	0	0.000
PortScatho	Portscat	13	Jun-06			136	136	11.63	7.915	0.675	0.640	−0.055	0.028
Plymouth	Ply	14	Oct-00	9	52[a]	2	63	10.25	8.129	0.645	0.633	−0.018	0.040
Start Point	StartP	15	Jul-06	62[a]	71[a]		133	11.75	8.084	0.643	0.649	0.009	0.007
Lyme Bay	Lyme	16	Jul-07			52	52	9.88	8.504	0.656	0.645	−0.016	0.027
Swanage	Swan	17	Jun-06	44[a]	11		55	9.50	7.97	0.645	0.672	0.041	−0.016
Brighton	Brighton	18	Sep-07			65	65	9.63	7.78	0.625	0.648	0.035	0.022
Hastings	Hast	19	Aug-00	5	67[a]		72	10.25	8.23	0.601	0.665	0.097[b]	−0.015
Hastings	Hast	19	Oct-06	54[a]	108[a]		162	12.25	8.32	0.628	0.645	0.026	0.019
North Sea													
Harwich	Har	20	Jun-00	47[a]	15	1	63	9.88	8.20	0.688	0.665	−0.033	−0.014
Harwich	Har	20	May-05	101[a]	58[a]		159	13.13	8.47	0.644	0.656	0.019	0.001
Norfolk	Norf	21	Jun-00	39[a]	39[a]	2	80	11.13	8.23	0.641	0.652	0.017	0.014
Bridlington	Brid	22	Aug-01	44[a]	40[a]		84	10.75	8.22	0.629	0.652	0.036	0.010
Bridlington	Brid	22	Jun-06	50[a]	56[a]		106	11.63	8.15	0.628	0.652	0.036	0.010
Northumberland	North	23	Jun-00	48[a]	46[a]		94	11.25	8.39	0.659	0.653	−0.011	0.004
Northumberland	North	23	Sep-05	56[a]	9		65	10.50	8.34	0.612	0.647	0.054[b]	0.014
Orkney-Hoy	Ork-Hoy	24	Jun-02	43[a]	40[a]	15	98	11.25	8.26	0.649	0.643	−0.008	0.020
Orkney-Sanday	Ork-Sand	25	Jun-02			38	38	9.25	8.58	0.686	0.682	−0.006	−0.041
Shetland	Shet	26	Jun-07				48	9.63	8.09	0.672	0.665	−0.010	−0.017
Gulmarsfjord	Gulm	27	Jun-02	41[aa]	39[a]		80	10.00	7.82	0.627	0.651	0.037	0.014

[a]Samples included in sex-segregated analysis; [b]Exhibited significant deviations from Hardy-Weinberg equilibrium (HWE) expectations; [c]Exhibited values significantly different from expectations of a panmictic model

0.008 ± 0.013, which was similar to values based on the other 7 loci (mean pairwise F_{ST} excluding *Cpag6c4b*: 0.002 ± 0.004). All outlier tests (pairwise and global) excluding the Gulmarsfjord males reported no significant outliers (Fig. S3 in the Supplement). Collectively, these results indicate that, among the analysed samples, potential divergent selection effects at locus *Cpag6c4b* were only detectable in comparisons involving Gulmarsfjord males.

Neutral genetic structuring

Spatial/temporal homogeneity among North Sea samples

Among the North Sea samples, excluding the Gulmarsfjord males, all intra-sample pairwise tests of differentiation between sexes were non-significant. Additionally, for those sites with temporally repli-

Table 2. Estimated statistical power for detecting various true levels of brown crab *Cancer pagurus* population differentiation (F_{ST}) by means of Fisher's exact *G*-tests in pairwise comparisons involving various permutations of sample sizes relative to this study (n = 30: minimum sample size used in pairwise tests; n = 84: average sample size; n = 120: representative of larger sample sizes employed). Power is expressed as the proportion of simulations reporting statistical significance at the 0.05 level. **Bold** values denote values obtained for simulated F_{ST} = 0 (Type I error), non-bold denotes values obtained for simulated F_{ST} = 0.0025

	n = 30	n = 84	n = 120
n = 30	**0.049**/0.409		
n = 84	**0.065**/0.699	**0.047**/0.981	
n = 120	**0.055**/0.784	**0.048**/0.997	**0.062**/1

cated samples, all intra-site comparisons were non-significant regardless of the arrangement of samples (i.e. whether tests were performed on samples pooled or segregated according to sex or time). All pairwise tests between sites yielded non-significant results, regardless of intra-site pooling or partitioning strategies. Upon pooling samples according to site, all pairwise tests were non-significant (Table 3), as was global F_{ST} (F_{ST} = 0.001, p = 0.057). The corresponding multi-locus global *G*-test was significant (p = 0.02); however, this was due to a significant value at only one locus (*Cpag3d7*; p = 0.025), omission of which resulted in a non-significant global *G* (p = 0.066).

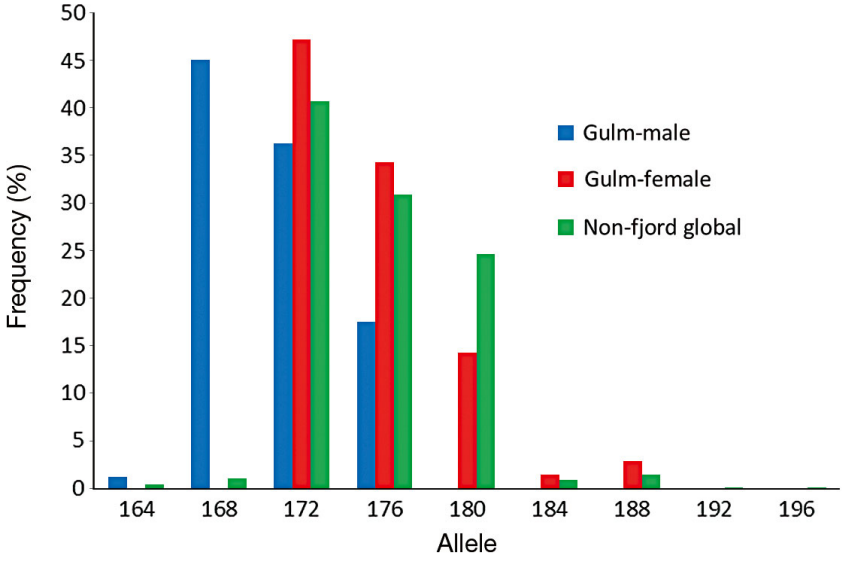

Fig. 2. Brown crab *Cancer pagurus* allele frequencies at microsatellite locus *Cpag6c4b* for Gulmarsfjord males, Gulmarsfjord females, and all other 'non-fjord' samples pooled

Genetic structuring within English Channel and Celtic/Irish Sea samples

Among the English Channel samples, no significant pairwise differentiation was detected between sexes in any samples. Significant temporal differentiation was reported between the Hastings 2000 and 2006 samples (Table 3), with this temporal differentiation also evident in pairwise comparisons between the relevant sex-segregated samples (Hast-2000 females vs. Hast-2006 males: F_{ST} = 0.0055, p = 0.045, exact *G*, p = 0.038; Hast-2000 females vs. Hast-2006 females: F_{ST} = 0.007, p = 0.031, exact *G*, p < 0.01). Significant differentiation between temporal replicates was reported for both within-bay samples from this region (Newlyn and Brittany), as well as for Jersey. Pairwise differentiation between temporal replicate samples within sites in many cases exceeded that between sites and contributed to an overall pattern of low but significant global structuring within the English Channel (global F_{ST} = 0.004, p = 0.001; global *G*, p < 0.0001), which did not show any consistent geographical or temporal pattern (see Table 3). Similar numerically small, yet significant, genetic structuring was also reported among Celtic/Irish Sea samples (global F_{ST} = 0.004, p = 0.001; global *G*, p < 0.0001), driven by the differentiation of, and among, the more southern samples in the region (Table 3).

Inter-regional genetic structure

The 2 samples from the west of Ireland (NWIre and GalBay) were not significantly differentiated from each other but exhibited a high proportion of significant pairwise test results against samples from other regions (Table 3). This differentiation was apparent in the PCoA (Fig. 3), which also highlighted the differentiation of the Saint Ives Bay sample (Celtic Sea) with 27 out of 31 significant pairwise tests (Table 3). Examining pairwise test results revealed that differentiation between samples was consistently small but in many cases significant, and did not follow a coherent spatial or temporal pattern but was rather similar to the spatial/temporal patchiness reported in the English Channel and Celtic/Irish Seas. This

Table 3. Pairwise F_{ST} values between all brown crab *Cancer pagurus* samples, with intraregional comparisons outlined, shaded and labelled. See Table 1 for sample codes. PF_{ST} and exact *G*-tests yielded similar patterns of significance, so only PF_{ST} are reported (*italics*: $p < 0.05$; underlined: $p < 0.01$; **bold**: $p < 0.001$)

	NWIre	Gal Bay	SEIre	Aber	Newq	SI Bay	Pen	New Bay-2000	New Bay-2007	Brit Bay-2000	Brit Bay-2006	Brit off-shore	Jer-2000	Jer-2007	Guer
	West of Ireland														
GalBay	0.003														
SEIre	0.003	**0.019**													
Aber	*0.007*	**0.018**	0.006												
Newq	0.004	**0.013**	0.005	–0.005											
SI Bay	0.004	**0.022**	**0.015**	*0.005*	0.005										
Pen	**0.013**	**0.024**	0.010	*0.005*	*0.004*	*0.004*									
NewBay-2000	0.010	**0.015**	0.011	0.007	*0.004*	**0.011**	0.002								
NewBay-2007	0.011	**0.017**	**0.021**	**0.013**	0.007	**0.012**	0.007	0.006							
Brit Bay-2000	**0.019**	**0.022**	**0.017**	0.008	0.007	**0.018**	0.008	0.009	**0.013**						
Brit Bay-2006	0.003	**0.014**	0.002	0.001	–0.001	0.003	0.002	0.005	0.008	**0.013**					
Brit offshore	*0.008*	**0.013**	0.011	*0.004*	0.001	0.005	0.002	0.001	0.002	0.002	*0.003*				
Jer-2000	0.013	**0.025**	0.007	0	0.002	0.008	0.001	0.001	**0.008**	0.004	0.002	0.001			
Jer-2007	*0.008*	0.009	0.012	0.007	0.002	0.008	*0.004*	0.006	*0.005*	0.009	0.003	0.002	0.008		
Guer	*0.008*	**0.011**	0.012	0.003	0.001	*0.006*	0.001	–0.001	*0.005*	0.009	0.001	0.001	0.002	0.003	
Portscat	*0.006*	**0.012**	0.012	0.001	–0.002	*0.004*	*0.003*	0.002	0.003	**0.011**	–0.001	0.001	0.001	0.003	–0.001
Ply	**0.023**	**0.019**	0.013	*0.007*	0.006	**0.019**	0.007	0.001	0.008	0.008	0.007	*0.004*	0.004	*0.006*	0.002
StartP	0.012	**0.018**	*0.009*	0.002	0.001	0.006	0	0.002	0.008	0.002	*0.004*	–0.001	0.001	*0.002*	0.001
Lyme	0.007	**0.020**	*0.011*	–0.001	0.003	0.004	*0.007*	*0.007*	0.014	**0.014**	0.002	*0.005*	0.003	**0.014**	0.003
Swan	0.007	*0.008*	*0.008*	**0.015**	*0.007*	0.011	0.002	*0.006*	*0.007*	0.009	*0.006*	0.002	0.009	0.002	0.004
Brighton	*0.009*	0.010	0.013	0.003	0.003	0.012	0.005	0.001	0.003	0.010	*0.004*	0.002	0.003	0.004	–0.001
Hast-2000	0.017	0.017	0.016	**0.011**	**0.008**	**0.022**	**0.011**	0.003	**0.012**	0.003	**0.013**	*0.005*	0.007	0.007	*0.004*
Hast-2006	*0.006*	0.013	0.012	0.002	0	0.003	0	0.003	0.006	0.008	0	0.001	0.003	0.002	0
Har-pooled	*0.006*	**0.011**	*0.008*	0.002	0	**0.007**	0.002	0.001	**0.007**	0.007	*0.002*	0.001	0.002	0.004	–0.001
Norf	**0.018**	**0.017**	0.011	*0.009*	0.008	**0.022**	**0.008**	0.002	**0.016**	*0.005*	**0.014**	0.006	0.003	**0.011**	0.003
Brid-pooled	0.011	0.009	0.014	*0.004*	0.005	**0.014**	**0.009**	*0.003*	**0.012**	0.007	0.007	*0.003*	0.005	0.008	0.002
North-pooled	0.011	**0.017**	0.009	0.003	0.002	**0.014**	0.002	0.001	**0.011**	*0.004*	0.005	0.001	0.001	0.005	0.001
Ork-Hoy	0.012	**0.015**	*0.008*	0.003	*0.004*	**0.012**	0.003	0.002	**0.012**	*0.005*	*0.003*	*0.003*	0.001	0.005	0.001
Ork-Sand	0.001	0.006	0.009	0.005	0	*0.006*	0.002	–0.002	–0.001	**0.013**	0	–0.001	0.004	0.003	–0.002
Shet	0.007	0.013	*0.008*	0.004	–0.007	*0.007*	0.002	–0.001	0.003	0.003	0.003	–0.003	0.002	0.003	–0.002
Gulm-females	0.003	0.004	0.005	0.004	0.002	0.012	0.005	–0.001	*0.008*	*0.010*	0.002	0.004	*0.007*	0.002	–0.001
Gulm-males	**0.028**	**0.035**	**0.037**	**0.028**	**0.026**	**0.036**	**0.030**	**0.027**	**0.036**	**0.029**	**0.030**	**0.025**	**0.032**	**0.027**	**0.024**

Table 3 (continued)

	Port-scat	Ply	StartP	Lyme	Swan	Brigh-ton	Hast-2000	Hast-2006	Har-pooled	Norf	Brid-pooled	North-pooled	Ork-Hoy	Ork-Sand	Shet	Gulm-females
Portscat																
Ply	*0.004*															
StartP	*0.002*	*0.004*														
Lyme	0.001	0.011	*0.007*													
Swan	0.008	**0.012**	*0.004*	**0.016**												
Brighton	–0.003	0	*0.004*	*0.007*	0.010											
Hast-2000	0.009	0.002	0.003	**0.015**	0.009	0.003										
Hast-2006	–0.005	0.005	0.001	0.003	0.005	0.003	0.009									
Har-pooled	0.001	*0.004*	0.001	0.003	*0.004*	0.002	**0.007**	0								
Norf	0.008	0	0.005	0.009	**0.011**	*0.006*	0.004	0.008	0.004							
Brid-pooled	0.003	0.005	0.003	*0.005*	0.009	0.003	*0.004*	0.005	0.001	0.003						
North-pooled	*0.003*	0.003	0	*0.005*	0.005	0.003	0.005	0.001	–0.001	0.003	0					
Ork-Hoy	*0.003*	0.001	0.001	*0.006*	*0.005*	0.003	*0.005*	0.002	0	0.001	0.001	–0.002				
Ork-Sand	–0.002	0.005	0.002	0.004	0.001	–0.002	0.005	–0.001	–0.001	0.004	0.002	0.001	0.003			
Shet	0	0.001	–0.001	0.005	0.004	–0.002	–0.002	0.002	–0.001	0.004	0	0	0.002	–0.001		
Gulm-females	0.001	0.001	0.002	*0.009*	0.004	–0.004	0	0.002	0	0.005	0.001	0.002	0.001	–0.005	–0.003	
Gulm-males	**0.028**	**0.035**	**0.025**	**0.032**	**0.032**	**0.025**	**0.029**	**0.026**	**0.027**	**0.030**	**0.026**	**0.026**	**0.026**	**0.027**	**0.024**	**0.027**

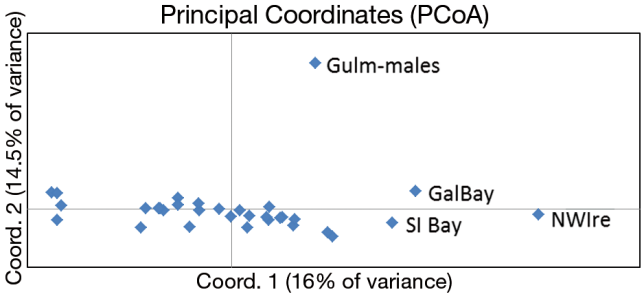

Fig. 3. Principal coordinates analysis of multi-locus pairwise F_{ST} for brown crab *Cancer pagurus*. See Table 1 for sample codes

Table 4. Analysis of molecular variance (AMOVA) in brown crab *Cancer pagurus* across the 3 main sampling regions (Celtic/Irish Sea, English Channel, North Sea), using either all data or sex-segregated data (see Table 1), and temporal samples within sites pooled or unpooled. F_{CT}: genetic variance among regions; F_{SC}: genetic variance among samples within regions

Temporal samples within sites	F_{CT}	p-value	F_{SC}	p-value
All individuals				
Unpooled	0.001	<0.001	0.003	<0.001
Pooled	0.001	<0.001	0.002	<0.001
Males only				
Unpooled	0.002	0.010	0.002	0.040
Pooled	0.002	<0.001	0.002	0.019
Females only				
Unpooled	0.001	0.059	0.003	<0.001
Pooled	0.001	0.018	0.0018	0.012

patchiness was not apparent in the STRUCTURE analysis, which reported unanimous support for a model of $K = 1$ in all analyses excluding the Gulmarsfjord males. Concordant with the lack of spatial or temporal patterning revealed by pairwise tests, no significant IBD was detected (all test results $p > 0.1$) and AMOVA reported greater variation among samples within regions than between regions (Table 4). AMOVA revealed similar patterns among partitioned males and females, and randomisation tests indicated no significant differences between the sexes for a number of indices (Table 5).

DISCUSSION

The present study represents the most geographically extensive investigation of brown crab population genetic structure to date. The research employed fine- and regional-scale spatial and temporal sampling, along with combined gene flow and kinship-based analyses and marker neutrality tests to elucidate the mechanistic underpinnings and eco-evolutionary significance of patterns of genetic diversity (following recommendations by Waples 1998, Nielsen et al. 2009, Iacchei et al. 2013). A striking feature of the results was the differentiation of the Gulmarsfjord males from all other samples, including females collected at the same site. This differentiation was driven by a single locus (*Cpag6c4b*), which was found to have a significantly higher F_{ST} than expected under neutrality in all pairwise comparisons involving this sample, suggesting divergent selection effects. Excluding the Gulmarsfjord males, all loci (including *Cpag6c4b*) conformed to neutral expectations and revealed numerically small but statistically significant differentiation among samples across the NE Atlantic. This global genetic structuring did not fit to an IBD model or an obvious hierarchical geographic pattern. Pairwise tests of differentiation (F_{ST} and exact G) revealed that the majority of comparisons were non-significant, including comparisons between geographically distant sites, but that a substantial number of comparisons exhibited significant differentiation that conformed to a model of chaotic genetic patchiness (CGP) in the sense that temporal and/or fine-scale differentiation often exceeded that at larger spatial scales (Johnson & Black 1984, Hedgecock 1994, Selkoe et al. 2006, Banks et al. 2007). Particular samples (discussed below) were associated with a high proportion of significant pairwise tests, indicating geographically local or sample-specific effects. Overall, patterns of neutral genetic variation in brown crab indicate local and unstructured genetic differentiation occurring against a background of high gene flow throughout the studied region.

The positive outlier status of locus *Cpag6c4b* in all comparisons involving the Gulmarsfjord males suggests that this locus, or a linked genomic region, is subject to divergent selection effects. This result

Table 5. Comparative analysis of genetic diversity indices for brown crab *Cancer pagurus* females and males, and corresponding p-values from 2-tailed tests

	Female	Male	Two-tailed p
A_R	7.709	7.755	0.674
H_o	0.643	0.632	0.330
F_{IS}	0.012	0.032	0.186
F_{ST}	0.003	0.003	0.638
r_{qg}	0.007	0.005	0.611

adds to a number of studies reporting selection effects apparent at microsatellite loci that were at some stage assumed to be neutral (Larsson et al. 2007, Skarstein et al. 2007, Westgaard & Fevolden 2007, Nielsen et al. 2009, Gaggiotti et al. 2009, White et al. 2010). Excluding locus *Cpag6c4b*, the Gulmarsfjord male sample was not significantly differentiated from the Gulmarsfjord female sample, or from most other samples. There are a number of potential explanations for such a pattern of locus-specific genetic differentiation. For example, the pattern could be generated without reproductive isolation through selection on individuals during early life stages followed by random mating each generation (i.e. differential genotype selection within a panmictic gene pool). At the other end of the spectrum, the pattern may reflect temporally stable reproductive isolation that is not detectable at neutral loci that lack the statistical power and/or are not at migration-drift equilibrium (Nielsen et al. 2009). Morphological, biochemical and genetic studies have demonstrated population differences among fjords, and between fjords and coastal areas, for a number of taxa (e.g. Jørstad & Nævdal 1989, Suneetha & Naevdal 2001, Oresland & Andre 2008, Teacher et al. 2013). While the allele frequency differences between brown crab sexes within the Gulmarsfjord may indicate gender-specific selection, mechanical mixing of individuals from differently adapted populations could also explain the differences. Evidence of female reproductive migration and lack of return migrations (Ungfors et al. 2007) suggests that the sampled Gulmarsfjord females may be allochthonous, while the male sample is composed of local (at least post-settlement) individuals. The differentiation between the sexes may therefore reflect allele frequency differences, and by extension adaptive differences, in their respective parental populations and not necessarily differential selection between sexes *per se*. In this sense, the pattern could be considered similar to the mechanical mixing of differently adapted migratory northeast Arctic cod (NEAC) and sedentary Norwegian coastal cod (NCC) populations within fjords (Sarvas & Fevolden 2005, Fevolden et al. 2012). The Gulmarsfjord crab data are consistent with other studies indicating that features of fjords may drive local adaptation (Dick et al. 2014), with both salinity and depth highlighted as candidate features by Fevolden et al. (2012), and identified as drivers of selection in other systems (salinity, Nielsen et al. 2009; depth, White et al. 2010). Future analysis of larval recruits would provide a means to investigate the relative roles of pre- and/or post-settlement selection and dispersal in shaping the observed pattern, while identification of underlying functional genetic differences and detection of other samples with similar adaptive fingerprints may help elucidate the environmental drivers.

The seemingly paradoxical pattern of CGP within broad-scale genetic homogeneity reported here has also been documented in a variety of marine species (limpets, Johnson & Black 1984; fish, Planes & Lenfant 2002, Selkoe et al. 2006; barnacles, Veliz et al. 2006). Cautious interpretation of such patterns is recommended, as when differentiation is low, multiple sources of artificial variance such as unrepresentative sampling (e.g. family/kin sampling, Hansen et al. 1997, Waples 1998, Waples & Gaggiotti 2006) and statistical noise (Waples 1998, Hedrick 1999, 2005) can be important and lead to false conclusions. Kin aggregation is generally assumed to be a transient phenomenon limited to newly settled recruits with little detectable signal in adult populations (Flowers et al. 2002, Planes et al. 2002, Selkoe et al. 2006, but see Iacchei et al. 2013), and as the analysed samples consisted of mixed cohorts of adults, kin aggregation would be an unlikely source of error. Furthermore, mean kinship values provided no strong evidence of large proportions of closely related individuals within samples. POWSIM analysis also indicated that the sample sizes conferred low probability of Type I errors. Therefore, while the genetic differentiation only amounts to slight differences in allele frequencies that may not have substantial evolutionary effects (Waples 1998), they nonetheless signal changes in the composition that may be a useful tool for better understanding recruitment dynamics and connectivity in this species (Selkoe et al. 2006, Knutsen et al. 2011).

Fine-scale genetic patchiness against a background of high gene flow has been variously attributed to 3 phenomena that may act in concert: large variances in individual reproductive success (sweepstakes recruitment), limited mixing of larvae from genetically different sources (larval cohesion) and local selection (Larson & Julian 1999). Sweepstakes recruitment has been reported for a number of highly fecund (Type III) marine taxa, such as brown crab, and may generate temporal or spatial genetic differentiation despite gene flow when recruitment is variable. Even in the absence of genetically isolated source populations, as might be the case here, larval cohesion (Selkoe et al. 2006) may enhance (Waples 2002) and be effectively indistinguishable from sweepstake effects (Turner et al. 2007). The divergent selection effect suggested for Gulmarsfjord

males highlights the potential for fine-scale selection in brown crab. However, outlier tests excluding this sample showed no evidence of selection effects for any other locus or sample combination. Furthermore, the temporal differentiation at a number of sites supports more prominent roles for processes like sweepstakes recruitment or larval cohesion as components of recruitment variability, rather than consistent selection effects. For example, the genetic patterns reported for the Hastings samples (the most eastern site sampled in the English Channel) are readily compatible with the proposed relationship between recruitment variability and genetic patchiness. This area was identified *a priori* as a potential hotspot of recruitment variability (D. Eaton unpubl. study on larval abundance and modelling). As the signatures of such processes are predicted to be diminished by post-larval dispersal (Planes & Lenfant 2002), the genetic patchiness observed here must be considered a conservative reflection of the extent of recruitment heterogeneity.

The CGP in brown crab was unusual in exhibiting a geographic pattern. In contrast to substantial numbers of significant differences among samples within the Irish/Celtic Seas and the English Channel, when the Gulmarsfjord males were excluded there were no significant differences among North Sea samples (see Table 3), which together with the spatial/temporal genetic homogeneity among Scandinavian samples described by Ungfors et al. (2009) indicates an absence, or lower level, of genetic patchiness in the North Sea compared to other regions studied. While such structuring likely reflects complex interactions between life history and environmental variables, the geographic pattern permits identification of specific factors that may be involved. North Sea brown crabs are significantly smaller than English Channel crabs (Pawson 1995), and as brown crab fecundity is linked to female size (Edwards 1979, Ungfors 2007), lower fecundity of North Sea crabs may reduce the potential extent of reproductive skews compared to those in the English Channel. For example, Palero et al. (2011) suggested that the selected harvesting of large females reduced variance in reproductive success in *Panulirus elephas*. McKeown & Shaw (2008b) posited the genetic monogamy of female brown crab as another life history feature that may increase variance in reproductive success among individuals, but they only analysed samples from the English Channel (which exhibited CGP). Multiple paternity has been reported in a number of closely related species (e.g. Jensen & Bentzen 2012) and may occur in brown crab from other areas, wherein it could serve to reduce variance in reproductive success among males. Seascape factors may directly influence, or interact with the genetic signatures of variance in reproductive success (Banks et al. 2007). The English Channel exhibits a higher degree of fine-scale oceanographic complexity and coastal heterogeneity in comparison to the North Sea (no CGP), both of which are factors that have been linked to localised sweepstakes recruitment in sea urchins (Banks et al. 2007). Likewise, the high proportion of significant pairwise tests reported for samples collected within semi-enclosed bays (Newlyn, Brittany, Galway, St. Ives) suggests an association between genetic differentiation and habitat structure as an additional component of fine-scale seascape structuring (Selkoe et al. 2010). Comparative studies among taxa with common and contrasting life history strategies will be necessary to elucidate the specific drivers of genetic variation (e.g. Selkoe et al. 2010); however, the brown crab data highlight the fact that genetic structuring may be driven by factors other than dispersal.

Pairwise tests reported a general pattern wherein the west of Ireland samples were differentiated from all samples except those collected in the northern North Sea. This may reflect geographically coherent connectivity. Sotelo et al. (2008) reported spider crab from the west of Ireland to be genetically distinct from more southern samples. However, in general, the genetic patterns for brown crab cannot readily be interpreted in the context of population connectivity/isolation. While the described factors driving CPG may lead to an underestimation of migration rate (m), changes in dispersal behaviour between different life stages may result in broad-scale genetic homogeneity masking of spatial gene flow restrictions (e.g. Berry et al. 2012). Partitioned analysis of sexes provided no evidence of greater structuring among male crabs that might be indicative of temporally stable spatial patterns of larval self-recruitment. Furthermore, the difficulties involved with deriving quantitative estimates of gene flow and dispersal from subtle genetic structure among large populations (Whitlock & McCauley 1999, Palsbøll et al. 2007, Hellberg 2009), and the discrepancy between levels of gene flow needed to limit genetic differentiation and dispersal needed to replenish stocks (Hauser & Carvalho 2008) are fundamental issues. Therefore, while the low level of genetic structure throughout the studied region is compatible with high gene flow, it cannot be ruled out that there is significant isolation of stocks on timescales of interest to management. Resolution of such spatial stock structure may be beyond the level of neutral genetic markers and

benefit from complementary analysis of markers under selection (Canino et al. 2005).

This study has implications for sustainable management of the brown crab fishery. The detection of adaptive diversification should enhance appreciation of local adaptation as a component of species biodiversity, and highlights a potential danger of indiscriminate harvesting of differentially adapted units on local scales. Stochastic recruitment variability suggested to underpin genetic patchiness may decrease resilience of local stocks to fishing and increase unpredictability in recovery (Kuparinen et al. 2014), and will necessitate a tailoring of the spatial scale of management (spatial bet hedging) according to biological and physical drivers of such recruitment variability. This study provides a baseline for future genetic studies of brown crab, which are needed in order to understand recent events such as expansions in census population size within the English channel (Molfese et al. 2014), and further demonstrates how intraspecific biodiversity and population viability is influenced by complex species–environment interactions other than dispersal.

Acknowledgements. This research was funded by DEFRA. We are indebted to all the fishers and CEFAS personnel who helped in collecting samples, particularly Derek Eaton and Julian Addison (both CEFAS).

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Editorial responsibility: Per Palsbøll, Groningen, The Netherlands

Submitted: August 22, 2016; Accepted: January 2, 2017
Proofs received from author(s): February 17, 2017