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Understanding marine reserve function in a seascape genetics context: *Nucella lapillus* in Strangford Lough (Northern Ireland) as an example

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ABSTRACT: A conservation priority in the marine environment is the establishment of ecologically coherent reserve networks. Since these networks will integrate existent reserves, an understanding of spatial genetic diversity and genetic connectivities between areas is necessary. Using Strangford Lough marine nature reserve (MNR) as a model, spatial genetic analyses were employed to evaluate the function of the lough. Samples of the marine gastropod *Nucella lapillus* (L.) from 7 locations in the reserve and adjacent areas were screened at 6 microsatellites. Genetic variation was temporally stable. Significant genetic structuring (F_{ST} = 0.133) was observed among samples. Genetic divergence and isolation by distance indicated reduced gene flow between the marine reserve and coastal samples relative to that between adjacent coastal samples. Partitioning of genetic variation between the reserve and coast was significant (AMOVA, 7.45%, $p < 0.005$). Samples within the reserve were not homogeneous, and genetic diversity decreased away from the mouth of the lough. Relative genetic isolation and reduced genetic diversity in the reserve may be caused by hydrographic barriers. A cline in genetic structure was observed in the Irish Sea. This is consistent with patterning of gene flow by predominant currents, habitat availability and seasonal oceanographic features. It appears that mesoscale topographic and hydrographic features drive patterns of genetic diversity, and these factors are likely to be important for understanding MNR function. For direct developers lacking a planktonic phase, such as *N. lapillus*, considerations of the ecological coherence of a network are likely to be highly dependent on the habitat lying outside reserves.

KEY WORDS: Ecological coherence · Dogwhelk · Microsatellite · Coastline · Dispersal

INTRODUCTION

Marine nature reserves (MNRs) or marine protected areas (MPAs) are an accepted and increasingly prevalent tool for the management and conservation of marine resources (Halpern & Warner 2002, Johnson et al. 2008a). A number of reserve programmes aspire towards ecologically coherent networks; these include the European Community's Habitats Directive (as special areas of conservation [SACs] within the 'Natura 2000' network) and the OSPAR network of MPAs for the North East Atlantic. A robust network of MPAs depends upon the individual area being self-sustaining, and on the connectivity dynamics of the entire network through dispersal processes (e.g. Halpern & Warner 2002). An understanding of the contemporary links among MPAs through dispersal is therefore fundamental for marine reserve design (Palumbi 2003, Cowen et al. 2006). Simulation models and site-selection algorithms have been applied in an attempt to determine appropriate spacing and size for marine reserves (e.g. Kaplan et al. 2006). Due to a poor understanding of the interactions between dispersal and oceanographic features, however, the design of marine reserves remains a difficult challenge. The wide range of dispersal scales for marine organisms means that

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reserve networks can never achieve a spacing that will suit all species. For example, half of the proposed marine sites in the Atlantic region of the Natura 2000 network could be unsuitable for species with intermediate dispersal scales (2 to 20 km; Johnson et al. 2008b). Furthermore, interactions among populations from reserves and non-reserve areas also underpin ecological coherence (Review of Marine Nature Conservation Working Group 2004). Population genetic studies of a range of species with varying dispersal capabilities are required to understand local patterns of genetic diversity, the proportion of local diversity a reserve is protecting and the dependencies between protected and non-protected habitat.

In addition to species-specific life history traits, the dispersal of marine organisms is affected by hydrographic processes across a range of scales. Topographic features, such as bays and headlands, create a variety of hydrographic phenomena (for review see Largier 2003). Frontal systems, oceanic currents, estuarine circulation, gyres and eddies have all been shown to restrict dispersal and gene flow (e.g. Bucklin et al. 2000, Shaw et al. 2004) and generate steep coastal marine clines (Hare et al. 2005). Furthermore, water and larval retention can occur in semi-enclosed marine systems such as sea loughs, fjords and embayments (e.g. Jessopp et al. 2007). Genetic studies of organisms living in fjordlands have shown that hydrographic and physical barriers impede gene flow and result in genetic structuring even in planktotrophic species with high dispersal capability (Sköld et al. 2003, Perrin et al. 2004 and references therein). Similarly, substantial levels of population genetic structuring and isolation have been observed in rafting gastropod species in small and very isolated sea loughs (Bell & Okamura 2005).

In the present study, we used Strangford Lough as a model MNR and employed microsatellite markers and spatial genetic analyses of the dogwhelk *Nucella lapillus* (L.) to evaluate the function of the lough. *N. lapillus* is a common intertidal gastropod mollusc restricted to rocky shores and found along most coastal environments of the North Atlantic (Fretter & Graham 1985). It has an oviparous mode of development, which lacks a planktonic larval phase. The reduced mobility of adults should imply low dispersal ability with rafting possibly only occurring during chance events (Highsmith 1985). Thus the restricted dispersal capability of this species lends itself to its suitability as a model species to test population connectivity. The alternative hypotheses of the present study were (1) that the MNR represents a random subsample of coastal genetic diversity (no more isolated or impoverished than any other area—effectively a null hypothesis) and (2) mesoscale topographic and hydrographic features drive patterns of genetic diversity such that they could be important for understanding MNR function.

MATERIALS AND METHODS

Study site. Strangford Lough in Northern Ireland (54° 23′ N; 5° 32′ W) was designated as an MNR in 1985 and as an SAC in 2006. It supports a considerable number of species (>2000) over a range of intertidal and subtidal marine habitats. It is an almost land-locked sea inlet with a surface area of 150 km^2 and a fjord-like gorge entrance. Strangford Lough was formed towards the end of the last glacial maximum (~20 000 yr ago), and it consists of a drowned drumlin field with many small islands, in addition to a long and highly contorted coastline some 240 km in length. From head to mouth, Strangford Lough is 30 km long and up to 8 km at its widest. The hydrodynamics of the lough are complex. Briefly, strong tidal currents (18 km h^{-1}) enter from the Irish Sea via the Narrows, an 8 km long channel (0.5 km wide) and then dissipate rapidly over the large shallow basin, which is mostly at 10 m depth or less. The flushing time of Strangford Lough is estimated to be between 3 and 4 d (Ferreira et al. 2007, Jessopp et al. 2007).

Sample collection, genomic DNA isolation and microsatellite typing. Samples were collected from 7 sheltered rocky shores inside Strangford Lough MNR, and away from the mouth of the lough in both directions on the Irish Sea coast (Fig. 1). The presence of *Ascophylum nodosum* was used as an indicator of sheltered habitats, since this alga species does not occur on exposed shores. At each site, approximately 30 *Nucella lapillus* were collected along a transect at 1 m intervals at mid-high shore in the summer of 2005. Ballyhornan (see Fig. 1) was revisited in 2006 for a temporal comparison of inter-annual genetic stability. Samples were stored at –20°C until further processing for DNA isolation. Genomic DNA was isolated from the foot muscle using the Nucleon® PhytoPure® kit (Tepnel) with slight modifications. Resulting DNA was diluted to 25 ng μl^{-1} and stored in TE buffer (pH 8.0) at –20°C. Genetic variation of *N. lapillus* was assessed at 6 microsatellite loci, each with di-nucelotide repeat motifs: *Nlw14*, *Nlw11*, *Nlw8*, *Nlw3*, *Nlw5* and *Nlw27* (Kawai et al. 2001). Polymerase chain reaction (PCR) conditions and annealing temperatures sometimes differed from those published (Table 1). *Nlw5* and *Nlw8* were amplified with a touchdown PCR with shortened cycling steps (35 s) to reduce stuttering. PCR reactions were undertaken in 12 µl reaction volumes containing 50 ng DNA, 100 µM dNTPs, 1× PCR reaction buffer (Invitrogen), 0.5 U *Taq* DNA polymerase (Invitrogen) and primer and $MgCl_2$ concentrations as in Table 1.

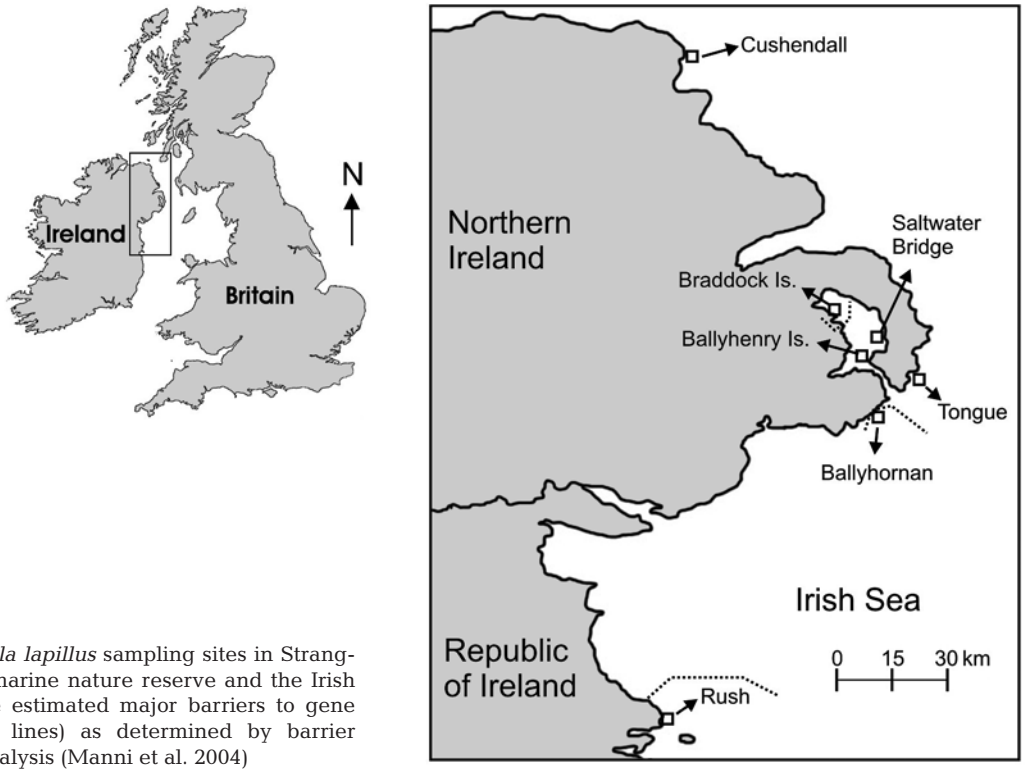

Fig. 1. *Nucella lapillus* sampling sites in Strangford Lough marine nature reserve and the Irish Sea, and the estimated major barriers to gene flow (dotted lines) as determined by barrier analysis (Manni et al. 2004)

Three microsatellite primers were re-designed with M13R tail extensions (see Table 1). PCR reactions in these cases, in addition to the locus primers (i.e. 1 locus specific-no tail and 1 locus specific with tail) also included the M13R IRD labelled primer in the following picomoles ratio: 1:0.25:1 (Schuelke 2000). Thermal cycling consisted of 5 min at 94°C, variable number of thermal cycles with 1 min at 94°C, 1 min at the optimal annealing temperature and 1 min at 72°C, followed by a final extension step of 5 min at 72°C (see Table 1 for locus-specific information). Following PCR amplification, 6 µl of stop solution (95% Formamide, 10 mM NaOH, 10 mM EDTA, 0.01% Pararosaniline) were added to reactions. PCR products were separated on 25 cm, 6% polyacrylamide gels mounted on a LI-COR 4200 automated system. Allele sizes were determined against a 20 bp DNA size ladder (Microzone™), loaded every 15 samples, using the SAGA genotyping software (LI-COR™). To minimise genotyping error, a control sample of known genotype was included in duplicate per 87 samples screened and a negative control was randomly performed. Replicate amplifications were performed on a subset of the samples to ensure repeatability and genotyping consistency.

Table 1. PCR conditions of the microsatellite loci described by Kawai et al. (2001) used in the present study. Tm (°C): annealing temperature; Primer (pM): primer concentration; $MgCl_2$: magnesium chloride concentration; Primer label: using IRD-labelled primer or M13-tail (see 'Materials and methods' for details)

Locus	Tm (°C)	PCR cycles (n)	Primer (pM)	$MgCl_2$	Primer label
Nlw11	57	24	0.5	1.5	IRD
Nlw3	57	23	0.5	2	IRD
Nlw5[a]	57	21	0.5	1.5	IRD
Nlw14	55	25	1	2.5	M13-tail
Nlw8[a]	57	19	2	1.5	M13-tail
Nlw27	52	24	2	2.5	M13-tail

[a]Indicates instances where touchdown PCR was used to amplify locus

Genetic variability. Summary sample statistics (mean and total number of alleles, expected and observed heterozygosity and allelic richness) were estimated using FSTAT 2.9.3.2 (Goudet 2001). Deviations from Hardy-Weinberg equilibrium (HWE; Guo & Thompson 1992) and genotypic linkage disequilibria were assessed using GENEPOP 3.4 (Raymond & Rousset 1995). Significance levels for multiple comparisons were adjusted using standard Bonferroni correction. Loci that deviated from HWE with heterozygote deficiencies were evaluated for the existence of null alleles in MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004). Null allele frequency was determined in FREENA 1.0 (Chapuis & Estoup 2007). Tests for recent reductions of

effective population size were carried out in BOTTLENECK 1.2.02 (Piry et al. 1999) using both the qualitative method based on a shift in frequency distribution of allele classes, and the Wilcoxon sign-ranked test, performed under the 2-phased model of mutation incorporating a stepwise mutation probability of 90%. Spatial patterns of allelic richness between MNR and coastal samples were investigated for north-south gradients by linear regression in Statview 5.0 (SAS Institute). Because null alleles can result in an underestimation of within-population genetic variation (Chapuis & Estoup 2007), the comparison of mean estimates included the loci that conformed to HWE only, although trends for the full dataset were found to be the same.

Population structure analysis. Genetic divergence among samples was compared using unbiased F_{ST} estimates computed accounting for null alleles in FREENA (Chapuis & Estoup 2007). Significance of F_{ST} values was assessed by bootstrapping (95% CI, 5000 replications over loci) as implemented in FREENA. Heterogeneity tests for allelic frequency divergence among samples for all loci and sample pairs were performed by permutation (1000 replications) in FSTAT. Isolation by distance (IBD) was tested by regressing pairwise estimates of linearised $F_{ST,}$ (F_{ST} / [1 – F_{ST}]) between all sites against the natural logarithm of geographic distances. Geographic distance between sites (km) was defined as the shortest distance by water, and in each case was taken as the mean of 3 repeated measures estimated with an online tool at www.maps.marine.ie/. Significance of the correlation between genetic and geographical distances was evaluated with a Mantel test using GENEPOP. The partitioning of genetic variance among samples was examined with a hierarchical multilocus analysis of molecular variance (AMOVA) using the locus-by-locus option in Arlequin 3.01 (Schneider et al. 2000) with 10 000 permutations and the MNR and coastal samples as the main groups for comparisons. Population genetic structure was also investigated using the Bayesian model-based clustering approach in Structure 2.2 (Pritchard et al. 2000) considering the null genotypes as homozygous for a recessive allele as suggested by Falush et al. (2007). The most appropriate number of genetic clusters (K) explaining the data was determined by comparing log-likelihoods (lnPr[X/K]) inferred from multilocus genotypes for K = 1 to K = 7 (i.e. number of samples examined). Since the alternative method of Evanno et al. (2005) for estimation of K is only appropriate for scenarios of strong genetic differentiation (F_{ST} = 0.15 to 0.4; Waples & Gaggiotti 2006) greater than that observed in the present study, we elected not to use it. To ensure convergence of the data, the software was run 5 times for each value of K, using the following parameters: 10^6 repetitions (burn-in = 250 000 iterations) under the admixture ancestry model and the assumption of correlated allele frequencies among populations.

Seascape spatial genetic analysis. Landscape genetic analysis has been increasingly successfully used to identify spatial patterns of genetic structure and possible regions of discontinuous gene flow in both terrestrial and marine studies (Miller et al. 2006, Kenchington et al. 2009). These analyses are based on statistical procedures that rely primarily on patterns of inter-individual genetic and geographical variation (see Manel et al. 2003 for a review). Here we used 2 seascape genetic analytical approaches. The program Barrier 2.2 (Manni et al. 2004) was used to identify barriers to gene flow, based upon the analysis of F_{ST} and geographical distance matrices. Alleles in Space 1.0 (AIS, Miller 2005) was employed to generate a landscape shape plot to visualise spatial patterns of genetic distances over the study area, and hence to assist with the identification of population structuring. AIS analyses were performed with a range of grid sizes and interpolation parameters using residual genetic distances (see AIS manual for details). Spatial patterns of genetic structuring were visualised in the Irish Sea and inside versus outside Strangford Lough MNR separately with final interpolation parameters of 50 × 50 grid size and a distance weighting value of 1 and 0.5, respectively.

RESULTS

Microsatellite loci

In total, 242 *Nucella lapillus* from 7 geographical areas representing the Strangford Lough MNR and the adjacent coast, including a temporal sample, were genotyped at 6 microsatellite loci. Basic sample summary statistics are presented in Table 2. No linkage disequilibrium was observed between loci pairs. All 6 loci were polymorphic, and the total number of alleles per locus ranged from 6 (*Nlw3*) to 19 (*Nlw5*, *Nlw8*) with a mean ± SE of 12.7 ± 2.4 across all loci/samples. The total number of alleles over loci per sample ranged from 29 (Braddock Island) to 51 (Rush), with a total of 76 alleles detected overall. Private alleles (1 to 2 alleles per locus per sample) were present in all samples, Rush had the greatest number (N = 3). Mean allelic richness per sample ranged from 4.39 (Braddock Island) to 7.56 (Rush). Expected heterozygosity over all loci for each sample ranged between 0.47 (Saltwater Bridge) and 0.65 (Rush). Observed heterozygosity (H_O) per locus and across all loci was generally lower than expected in the majority of the samples, suggesting heterozygote

Table 2. *Nucella lapillus.* Summary statistics for samples screened for 6 microsatellite loci. Samples were collected in 2005 and are listed based on their geographic location from north to south (see Fig. 1). *N*: number of sampled individuals; *n*: number of reliable genotypes per locus; Allele range: size range of alleles in base pairs; *A*: observed number of alleles (number of private alleles in parentheses); *Rs*: allelic richness (calculated based on a minimum sample size of 18 diploid individuals); H_O: observed heterozygosity; H_E: expected heterozygosity; *R*: null allele frequency estimator (**bold**: significant deviations from Hardy-Weinberg equilibrium after standard Bonferroni correction). –: non-applicable

Locus	Cushendall	Tongue	Strangford Lough MNR			Ballyhornan	Ballyhornan (2006)	Rush	All
			Ballyhenry Island	Saltwater Bridge	Braddock Island				
Nlw11									
n	30	30	30	30	30	30	32	30	242
Allele range	160–168	158–176	158–168	160	156–176	156–184	156–184	156–178	156–184
A	4	7 (1)	5	1	4	10	12 (1)	8 (1)	15
Rs	3.6	6	4.3	1	3.4	8.4	10.3	6.8	8.4
H_O	0.300	0.267	0.233	–	0.100	0.200	0.156	0.233	0.186
H_E	0.613	0.458	0.276	–	0.523	0.760	0.791	0.728	0.519
R	**0.171**	**0.161**	0	0.001	**0.277**	**0.308**	**0.352**	**0.282**	0.194
Nlw14									
n	30	30	30	27	22	30	31	18	218
Allele range	182–196	182–196	180–186	178–186	182–184	184–188	184–188	182–196	178–196
A	5 (1)	4	4 (1)	4 (1)	2	3	3	4	8
Rs	4.1	3.5	2.8	3.5	2	2.5	2.4	4	3.8
H_O	0.167	0.433	0.100	0.037	–	0.067	0.097	0.056	0.120
H_E	0.328	0.366	0.098	0.179	0.091	0.129	0.095	0.510	0.225
R	0.152	0	0	**0.346**	0.528	0.100	0.151	**0.649**	0.241
Nlw3									
n	30	30	30	28	30	30	29	30	237
Allele range	180–186	180–188	180–186	180–186	180–182	180–188	178–184	178–186	178–188
A	2	4	4	4	2	5	4	4	6
Rs	2	3.6	3.6	3.6	2	4.2	3.2	3.6	3.9
H_O	0.233	0.333	0.400	0.500	0.300	0.433	0.276	0.533	0.376
H_E	0.306	0.418	0.564	0.540	0.259	0.518	0.348	0.448	0.425
R	0.067	0.025	0.110	0.176	0	0.040	0.259	0	0.085
Nlw5									
n	30	30	30	30	30	30	31	30	241
Allele range	178–220	176–218	174–220	174–220	174–222	162–218	174–218	176–220	162–222
A	9	11	11	13	11 (1)	13 (2)	13	15	19
Rs	6.8	9.6	9.1	11.2	9.7	10.4	10.7	13.6	11
H_O	0.700	0.800	0.667	0.767	0.800	0.900	0.839	0.867	0.792
H_E	0.659	0.825	0.751	0.765	0.741	0.829	0.842	0.916	0.791
R	0	0.017	0.040	0.005	0	0	0.064	0.005	0.017
Nlw8									
n	30	28	28	21	30	29	28	30	224
Allele range	139–163	135–161	143–167	143–167	137–171	137–169	139–167	137–173	135–173
A	9	9 (1)	9	7	7	9	11	15 (1)	19
Rs	8.1	7.1	7.4	7	6.2	8	9.4	13	10.8
H_O	0.633	0.393	0.464	0.762	0.333	0.828	0.536	0.933	0.610
H_E	0.752	0.525	0.509	0.812	0.559	0.808	0.763	0.920	0.706
R	0.076	0.206	0.169	0.370	**0.143**	0.063	0.272	0	0.162
Nlw27									
n	30	29	29	27	30	30	31	27	233
Allele range	142–154	142–156	142–156	146–154	148–154	146–156	146–156	142–160	142–160
A	4	6	8 (1)	4	3	5	5	5 (1)	9
Rs	3.2	5.5	7.3	4	3	4.5	4.3	4.4	5.5
H_O	0.167	0.655	0.655	0.741	0.567	0.233	0.419	0.296	0.467
H_E	0.217	0.599	0.735	0.595	0.659	0.356	0.386	0.273	0.477
R	0.064	0.077	0.104	0.143	0.041	**0.076**	0.088	0.235	0.104
Total									
N	30	30	30	30	30	30	32	30	242
Total *A*	33 (1)	41 (2)	41 (2)	33 (1)	29 (1)	45 (2)	48 (1)	51 (3)	76
Mean *Rs*	4.6	5.9	5.7	5	4.4	6	6.7	7.6	5.8
Mean H_O	0.367	0.480	0.418	0.454	0.366	0.441	0.385	0.521	0.429
Mean H_E	0.479	0.532	0.487	0.466	0.490	0.565	0.537	0.648	0.526

deficiency. Ten of 48 sample-locus combinations deviated significantly from HWE after a standard Bonferroni correction with no particular bias; thus we can exclude the possibility of population sub-structuring (i.e. a Wahlund effect). One locus (*Nlw11*) deviated from HWE in 6 of 8 samples and a second locus (*Nlw14*) in 2 of 8 samples. In all cases, there was evidence for heterozygote deficiencies. Allele frequencies for each locus in each sample are provided in Appendix 1.

Intrapopulation genetic diversity in MNR and coastal samples

Genetic diversity was highest for samples located south of Strangford Lough in comparison to those inside and north of the MNR (Table 2). There was a significant overall south-north decrease in allelic richness (p = 0.005, Fig. 2). Within Strangford Lough MNR, genetic diversity decreased with increasing distance away from the mouth of the lough. Ballyhenry Island, located near the Narrows, had the highest diversity (mean allelic richness (*Rs*) = 5.75, number of alleles (*A*) = 41), followed by Saltwater Bridge on the eastern shore (*Rs* = 5.06, *A* = 33). Braddock Island, the most northerly point sampled inside the lough, was depauperate (*Rs* = 4.39, *A* = 29) in comparison. A locus (*Nlw11*) was monomorphic in 1 instance only at Saltwater Bridge in the MNR. No significant differences in basic summary statistics were observed between the temporal samples from Ballyhornan (Table 2 and Appendix 1). This involved the comparison of samples comprising individuals from different age cohorts. Thus, it provides a realistic assessment for possible changes in genetic diversity over time, since it takes into consideration local population dynamics (i.e. death and recruitment). The current data suggest no evidence of a recent population bottleneck in any of the samples surveyed in the present study. Distributions of allele frequency classes did not deviate from expectations, and Wilcoxon sign-ranked tests for heterozygote excess were all non-significant (p > 0.05).

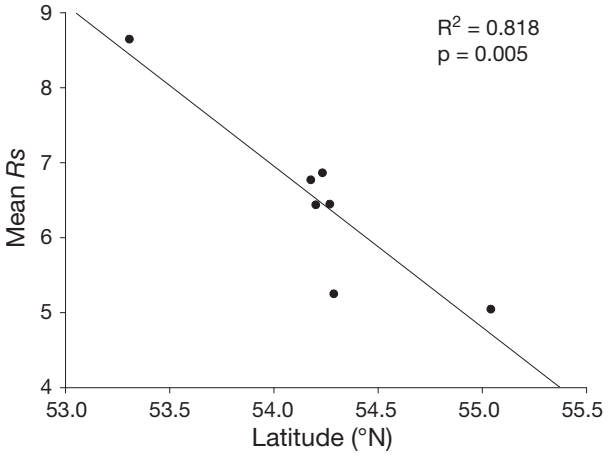

Fig. 2. *Nucella lapillus.* Linear regression of mean allelic richness vs. latitude estimated for the 4 loci in Hardy-Weinberg equilibrium

Population differentiation of MNR and coastal samples

Genetic differentiation over all 7 *Nucella lapillus* samples was significant with a global F_{ST} = 0.133 (95% CI = 0.065 to 0.219, p < 0.05) for all loci included. All pairwise F_{ST} comparisons for genetic differentiation were significant (p < 0.05) and ranged between 0.034 and 0.277 (Table 3). An inter-annual comparison of pairwise F_{ST} between Ballyhornan 2005 and 2006 was non-significant (F_{ST} = 0.009, 95% CI overlapped with 0, p > 0.05). All pairwise comparisons between sites for heterogeneity of allelic frequencies were highly significant with the exception of 2 comparisons involving Ballyhenry Island and Saltwater Bridge, and Ballyhenry Island and Tongue (Table 3, above diagonal). IBD was detected in *N. lapillus* (Fig. 3); there was a significant positive correlation between genetic and geographic distances (Mantel, R^2 = 0.341, p < 0.005). The slope of the line was greater between MNR and coastal areas only (excluding coast-coast and MNR-MNR comparisons; upper trend line, Fig. 3), compared to coastal comparisons (lower trend line) suggesting the exis-

Table 3. *Nucella lapillus.* Summary of tests for pairwise comparisons of sample differentiation based on allelic frequency differences at 6 microsatellite loci. Pairwise F_{ST} values are given below the diagonal and statistical significance of comparisons of allelic heterogeneity between samples above the diagonal. *p < 0.05; NS: non-significant

	North		Strangford Lough marine nature reserve			South	
	Cushendall	Tongue	Ballyhenry Island	Saltwater Bridge	Braddock Island	Ballyhornan	Rush
Cushendall	–	*	*	*	*	*	*
Tongue	0.098	–	NS	*	*	*	*
Ballyhenry Island	0.194	0.034	–	NS	*	*	*
Saltwater Bridge	0.244	0.091	0.047	–	*	*	*
Braddock Island	0.132	0.045	0.100	0.101	–	*	*
Ballyhornan	0.065	0.048	0.120	0.164	0.078	–	*
Rush	0.130	0.168	0.277	0.276	0.157	0.103	–

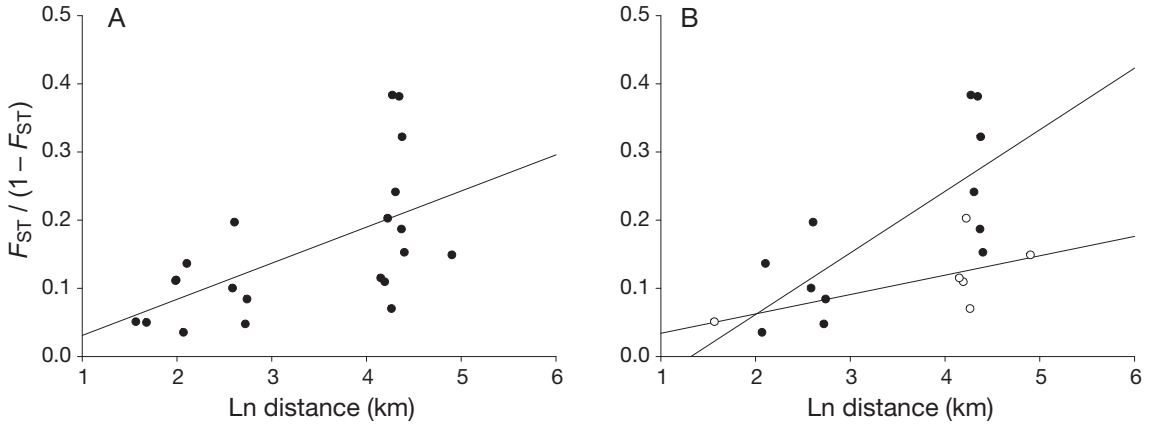

Fig. 3. *Nucella lapillus.* Relationship between genetic differentiation $F_{ST}/(1 - F_{ST})$ and geographic distance (ln-transformed) between population samples. (A) Trend line for all observations; (B) upper trend line indicating marine nature reserve (MNR)-coast comparisons only (●) and lower trend line indicating comparisons involving coastal samples only, i.e. excluding MNR samples (○)

tence of greater genetic subdivision between Strangford Lough MNR and coastal areas than between coastal areas only. Hierarchical AMOVA analyses showed significant differences in genetic partitioning of *N. lapillus* at all levels of geographic organisation: among groups, within groups and within samples (Table 4). Significant genetic differentiation was detected among the groups MNR versus Coast (Table 4). While 83.9% of the genetic variance was explained by the within-population component, 7.45% was due to genetic differences between Strangford Lough MNR and coastal samples. The MNR group was characterised by lower levels of genetic differentiation (5.96%, $p < 0.001$) than the coastal group (11.19%, $p < 0.001$).

The Bayesian model-based cluster analysis, without *a priori* definition of population structuring, supports the existence of 5 genetically distinct clusters. The proportional membership of individuals to each of the 5 inferred clusters is illustrated in Fig. 4. The MNR samples are predominantly represented by a mix of 2 clusters ('dark grey' and 'medium grey') closely resembling Tongue. Individuals from Cushendall and Rush primarily had membership to 'white' and 'black' clusters, respectively, while individuals from Ballyhornan were more or less equally spread among the 5 inferred clusters. Overall, the data suggest the existence of a north-south gradient easily noticeable by the reduction in the proportion of membership to the 'white' and 'black' clusters moving south of Cushendall and north from Rush, respectively. These 2 clusters are not particularly represented within the MNR, suggesting limited gene flow.

Barrier spatial analysis revealed 3 areas of genetic discontinuities, which are both indicative of reduced gene flow and are consistent with the patterns in the Bayesian cluster analysis. Thus, barriers of reduced gene flow were detected between Rush and Ballyhornan, Ballyhornan and the MNR and within the MNR between Braddock Island and the other MNR populations (Fig. 1). The genetic landscape shape analysis (Fig. 5) clearly emphasises the south-north cline in population genetic structure throughout the study area, alongside more local variations in genetic diversity. Three main discontinuities in gene flow were indicated by 3 large individual peaks in genetic distances on the surface plot (Fig. 5a, peaks 1, 2, and 3). One was observed between Rush and Ballyhornan, and the other 2 peaks occurred on either side of the mouth of Strangford Lough MNR, at Tongue and Ballyhornan. Although a trough between Ballyhornan and Rush was also detected with AIS, this was not corroborated with the Barrier analysis. Inside the MNR, a

Table 4. *Nucella lapillus.* Summary of analysis of molecular variance (AMOVA) among population samples. Degrees of freedom (df), variance components, percentage of the total variance explained by the groupings (%) and their significance are indicated

Source of variation	df	Variance components	% total variance	p
MNR vs. Coast				
Among groups	1	0.15	7.45	<0.005
Between samples within groups	5	0.16	8.69	<0.001
Within samples	413	1.55	83.86	<0.001
MNR				
Among MNR samples	2	0.09	5.96	<0.001
Within MNR samples	177	1.43	94.04	
Coast				
Among coastal samples	3	0.21	11.19	<0.001
Within coastal samples	236	1.64	88.81	

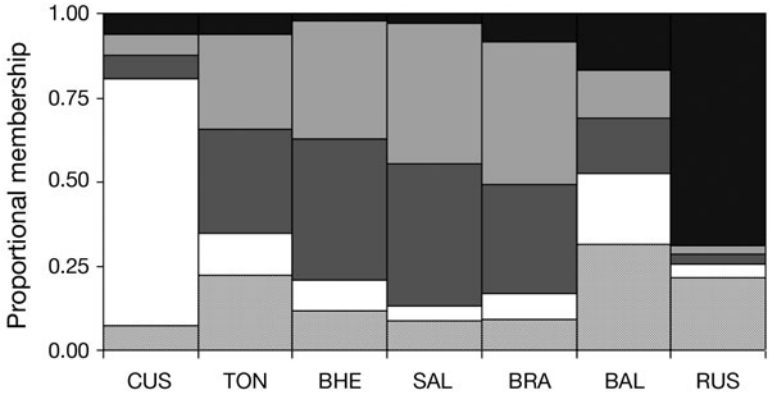

Fig. 4. *Nucella lapillus.* Summary of the proportional genetic membership of individuals from each sample to the inferred clusters (represented by different shades of grey) as determined by the Bayesian cluster analysis assuming $K = 5$ (see 'Results' for details). CUS: Cushendall; TON: Tongue; BHE: Ballyhenry Island, SAL: Saltwater Bridge; BRA: Braddock Island; BAL: Ballyhornan; RUS: Rush

smaller peak (Fig. 5a, peak 4) was observed between Braddock Island and the other MNR populations. Strangford Lough MNR and Ballyhornan and Tongue coastal samples were investigated separately in an attempt to emphasise the resolution of genetic structuring at smaller spatial scales (Fig. 5b). Excluding the high peak surrounding Braddock Island, overall there was a higher degree of genetic similarity (i.e. trough regions in the surface plot) among the MNR samples. While the coastal Tongue sample was characterised by a comparatively small peak in relation to other MNR samples, there was an obvious cline separating Ballyhornan from both Tongue and the other MNR samples.

DISCUSSION

The present study revealed that considerations of the genetic functioning of reserves will involve processes at a number of scales. Insofar as *Nucella lapillus* is concerned, Strangford Lough occurs along a cline of genetic diversity, but within that cline there are localised variations in gene flow and diversity. A combination of descriptive genetic statistics and analytical approaches supports the observation that *N. lapillus* populations in Strangford Lough MNR are not only genetically depauperate in comparison to adjacent coastal areas, but they are also comparatively isolated in terms of gene flow. This was particularly evident from the percentage of the total number of alleles (N = 76), which were lower in MNR samples (38.1 to 54%) in comparison to coastal samples (43.4 to 67%). Thus, the relatively reduced levels of allelic diversity indicate that the MNR does not fully represent all genetic diversity existing in the area. The reduced diversity levels within MNR populations are most likely related to a stronger effect of genetic drift acting upon a partially isolated gene pool. Despite the relatively low genetic diversity within the MNR, however, there were still private alleles within the lough.

Nucella lapillus lacks a planktonic phase, and during their lifetime (10 yr), individuals are unlikely to crawl more than 30 m (Fretter & Graham 1985). Thus, dispersal over longer distances possibly occurs by rafting or drifting in the water column (e.g. Highsmith 1985, Martel & Chia 1991). While this mode of development is suggestive of limited gene flow, an increasing number of investigations, including the present study, indicate that gene flow is substantially greater than expected (e.g. Colson & Hughes 2004, Bell & Okamura 2005). Based on our data, however, *N. lapillus* still exhibits significant genetic structuring (F_{ST} = 0.133, $p < 0.05$) over a very small spatial scale insofar as marine habitats are concerned (~150 km; 450 km shoreline distance). This level of genetic divergence (F_{ST} = 0.034 to 0.27), which was temporally stable, suggests that on an ecological timescale, there is limited connectivity between areas. Genetic divergence was in the range of published values both for *N. lapillus* (e.g. F_{ST} = 0.09 to 0.17; Colson & Hughes 2004, Bell 2008) and for planktotrophic species (F_{ST} = 0.00 to 0.17) but is less comparable with brooding or direct developing species (F_{ST} = 0.14 to 0.79; for review see Colson & Hughes 2004). The pattern of genetic structuring among *N. lapillus* populations in the Irish Sea was consistent with an IBD model, reflecting a stepping-stone mode of expansion, i.e. continuous migration between neighbouring areas. Colson & Hughes (2007), who investigated the post-glacial expansion of *N. lapillus*, did not detect IBD along the east Atlantic route. However, they sampled sparsely over a large geographic area and included only 2 samples from the Irish Sea. In the present study, although the geographic coverage was comparatively reduced, more samples were taken over smaller spatial scales. IBD over larger geographical scales might not be detected because of the confounding effects of more founders arriving by long-distance dispersal via rafting. These confounding effects should not be as noticeable on smaller geographical scales because continuous gene flow among neighbouring areas should lessen the effect. The results reported here concur with those of Bell & Okamura (2005), who also described IBD in *N. lapillus* from samples collected in the south-west coast of Ireland, Plymouth, Bristol and northern France. However, evidence for IBD in their study was only detected following exclusion of samples from Lough Hyne, Ireland.

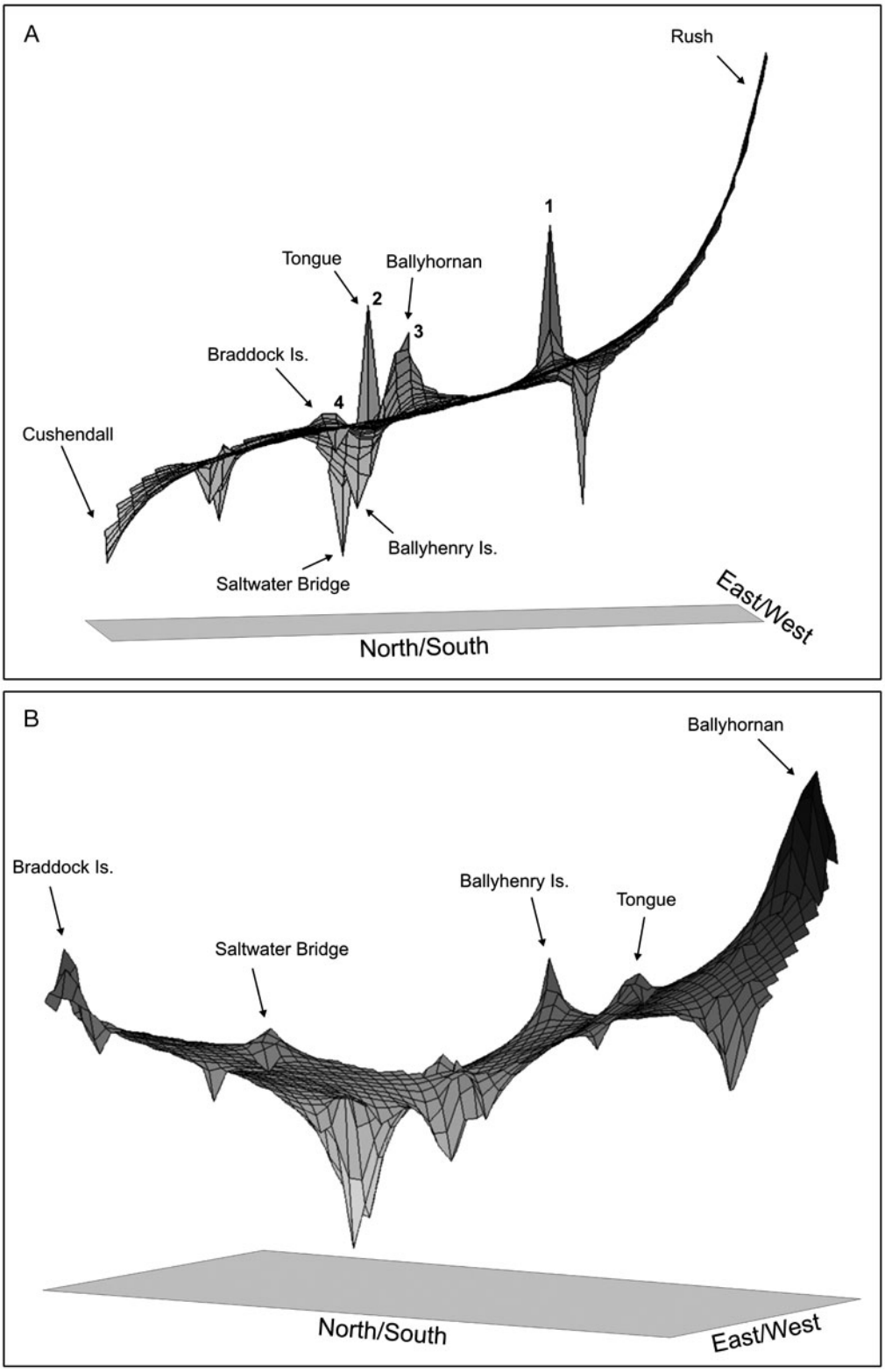

Fig. 5. *Nucella lapillus*. Genetic landscape shape analysis. The *x*- and *y*-axes correspond to geographical coordinates of individual samples, and the *z*-axis corresponds to genetic distance. Positive peaks (troughs) in the plot represent areas where genetic distances between individuals are large. These represent genetic discontinuities or possible barriers to gene flow. Conversely, negative peaks represent areas and high genetic similarities (low genetic distances). (A) All samples included; (B) marine nature reserve samples, Ballyhornan and Tongue. Identifiers (numbers) for the 4 main peaks are discussed in 'Results'

Spatial patterns of population genetic structuring and the detected genetic discontinuities suggest that local oceanographic factors and coastline topography have a major role in influencing the patterns of gene flow in *Nucella lapillus*. The direction of gene flow between samples can be deduced from the observed changes in the proportion of individuals from each sample that allocate to each of the 5 inferred clusters. Thus, it was apparent from the cluster analysis that gene flow occurs mostly with the predominant nearshore currents in a southerly direction. However, there appears to be little active migration north against the predominant southerly currents, but some gene flow does occur perhaps in line with offshore currents that flow north in the Irish Sea (Xing & Davies 2001). A north-south cline in genetic structure was evident from the steep slope between the most northerly and southerly points as identified in the landscape shape analysis. Predominant current directions may in part therefore explain the cline in genetic structure from south to north observed in the present study. Discontinuities in gene flow along the coast may be equally important in maintaining this cline and in further explaining why gene flow among northern samples is greater compared to southern samples. The seascape genetic analyses identified gene flow barriers at the mouth of Strangford Lough, within the MNR and between Ballyhornan and Rush. A possible explanation for this is linked to the presence of a non-uniform coastline and other topographic features such as headlands surrounding the mouth of Strangford Lough. These could potentially deflect nearshore oceanographic currents creating eddies and retention zones, thus explaining this gene flow discontinuity (e.g. Largier 2003, McCulloch & Shanks 2003). Furthermore, at the mouth of Strangford Lough, strong tidal currents leaving the Lough meet those of the Irish Sea and as a consequence, there is an exceptionally great amount of turbulence, which would also disrupt gene flow. This type of barrier to gene flow has been reported by Bell & Okamura (2005) in a study also involving *N. lapillus*. Gene flow was restricted between samples separated by a fast-flowing turbulent body of water (<250 cm s^{-1}) known as the 'Rapids' connecting Lough Hyne to the sea. Thus, turbulent water may also act as a barrier to rafting species by causing sinking or disintegrations of rafts.

The apparent barrier to gene flow between Ballyhornan and Rush could be due to habitat availability or coastal current patterns. Lack of suitable habitat has been shown to be a barrier to dispersal in at least one other oviparous marine mollusc lacking a planktonic stage (Allcock et al. 1997). The shore is mostly sand in the counties of Louth and Meath that lie between Rush and Ballyhornan (82% of 120 km of coastline according to Neilson & Costello 1999). This represents unfavourable habitat for *Nucella* that would limit stepping-stone dispersal. *N. lapillus* may have other habitat requirements that limit its population connectivity as has been observed for other rocky shore species (Ayre et al. 2009). The potential dispersal barrier formed by a lack of habitat and or requirements is also consistent with seasonal oceanographic features, which develop as the result of thermal stratification during the summer months (when most marine organisms are reproductively active). Frontal systems develop along much of the coast directly south of Ballyhornan and Strangford Lough in the Irish Sea and deflect nearshore currents offshore, thereby limiting gene flow (Xing & Davies 2001). Also in the western Irish Sea, south of Strangford Lough, thermal stratification above deep cold water troughs generates a cyclonic gyre, which can act as a retention mechanism for the juveniles of commercially important species (e.g. *Nephrops norvegicus*, *Gadus morhua*) (e.g. Hill et al. 1996) thus limiting gene flow and promoting genetic structuring as has been observed in other gyre systems (Bucklin et al. 2000).

Nucella lapillus genetic diversity declined towards the north. Similar to genetic structure, a cline in genetic diversity may also be maintained by the predominant southerly currents, which ensure there is a lack of gene flow from genetically diverse populations located in the south. Closer examination of the genetic relationships among *N. lapillus* samples considered in the present study suggests that gene flow is more restricted between Strangford Lough MNR and coastal samples than between coastal samples at similar spatial scales. Assessment of additional samples would be required to confirm these trends. Furthermore, gene flow is also limited within Strangford Lough. These findings are congruent with reports from other semi-enclosed marine areas where coastal configuration retains water and restricts gene flow (e.g. Bell & Okamura 2005). Genetic isolation has also been detected in planktonic-dispersing sea stars from semi-enclosed fjords (Sköld et al. 2003, Perrin et al. 2004). It was interesting to note that the levels of genetic differentiation among *N. lapillus* samples observed in the present study are similar to those reported by Bell & Okamura (2005) between samples from Lough Hyne and nearby coastal areas (F_{ST} = 0.15 to 0.25). This was unexpected, considering that Lough Hyne is a much smaller area (~400 ha) with a much lengthier flushing time (41 d, Johnson et al. 1995) and is considerably more physically enclosed compared to Strangford Lough. More importantly, the opening to the sea via the 'Rapids' in Lough Hyne is just 25 m in width compared to the 'Narrows' at Strangford Lough, which is 0.5 km wide. Thus, it is surprising that, given the much

wider channel width and greater tidal exchange of Strangford Lough, the overall levels of gene flow are nevertheless similar. Given the nature of rafting, the relative level of flushing may not be the important driver of genetic isolation for species such as *Nucella*.

Strangford Lough MNR populations were genetically depauperate compared to coastal populations located south of the lough in the Irish Sea. Contemporary gene flow into the MNR mainly occurs in the direction of the predominant currents from genetically depauperate northern areas. Thus, an influx of new alleles from the most genetically diverse populations located south of the MNR probably rarely occurs. The gene pool in the lough, while relatively reduced, was distinctive in that it contained private alleles. Genetic diversity was also not homogeneous among MNR populations, and diversity decreased with increasing distance away from the mouth of the lough, probably due to patterns of rafting and habitat availability within the lough. Within the MNR, there is a gradation from the Narrows, moving both into the body of the lough and offshore into the Irish Sea, from bedrock through cobble, gravel and sand to mud. Certainly in northern parts of the MNR, colonisation may be hampered perhaps due to a lack of suitable rocky shore habitat required by *Nucella lapillus*, thus maintaining small effective population sizes, which by default are more prone to the effects of genetic drift. Alternatively, genetic bottlenecks, i.e. reductions in genetic diversity following drastic reductions in effective population sizes, might explain lower genetic diversity levels in the MNR. However, this appears not to be the case as there was no evidence for genetic bottlenecks in any of the samples surveyed. Strong effects of genetic bottlenecks in *N. lapillus* may be difficult to detect, possibly due to rapid recovery of populations through gene flow and only slight reductions in genetic diversity in affected recolonised populations (see Colson & Hughes 2004). Lowered genetic diversity inside Strangford Lough MNR may reflect smaller effective population sizes inside the MNR (Frankham 1996). *N. lapillus* can also be affected by sterility due to imposex, i.e. the development of male secondary sexual characteristics in females. This can be induced in *N. lapillus* by exposure to the antifouling chemical tributylin (TBT; Gibbs & Bryan 1986) and sewage (Santos et al. 2008). Since the late 1980s, however, the use of TBT has been banned, and monitoring programmes between 1994 and 1996 revealed that Strangford Lough populations were not affected by high levels of imposex (DOE 1996). To our knowledge there are no reports indicating that the MNR experiences elevated sewage levels compared to coastal samples. The effect of imposex therefore cannot explain lower genetic diversity in Strangford Lough MNR populations compared to coastal populations.

Our results pose fundamental questions of what it means for a reserve to be ecologically coherent. This term implies that a reserve as part of a network should have the capacity to support other reserves and interact with surrounding habitat (Johnson et al. 2008b). In the case of Strangford Lough, the MNR is geographically isolated from its nearest European SACs, an island located off the north coast of Northern Ireland (Rathlin) and an area to the south (Murlough) that is mostly dominated by sandy habitat. These SACs are both too far away and, in the case of Murlough, lack suitable habitat and associated flora and fauna to be able to act as sources of rocky shore species such as *Nucella*. Thus for species with restricted dispersal and other habitat requirements, it is unlikely that the SACs and Strangford Lough MNR would interact. From the point of view of protecting the greatest genetic diversity of *N. lapillus*, Strangford Lough is unsuitable due to its genetic isolation and consequent reduced levels of variation. However, the distribution of other rare habitats located within Strangford renders it a regional priority for protection (JNCC 2006). There is no clear alternative candidate SAC in the vicinity of Strangford that could act as the local hotspot of genetic variation. The next most likely candidate SAC perhaps suitable for inclusion in the network in genetic diversity terms (Rush, a southern site) is separated from the other areas by habitat connectivity and/or hydrographic features. Thus, albeit suitable from a genetic perspective, Rush, due to its isolation, is unlikely to interact coherently with the other SACs in any case. The present study is therefore an example of how the habitat between SACs should be the focus for achieving ecological coherence. Maintaining the habitat suitability for outcrops of rock along otherwise sandy coastlines may therefore be crucial in maintaining coherence for rocky shore species with stepping-stone modes of population expansion. For a greater understanding of the functioning of marine reserves, a multi-species approach, taking into consideration variable life history strategies and habitat requirements, will be required for designating candidate SACs that realistically have the potential to maintain ecological coherence.

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Appendix 1. *Nucella lapillus.* Allele frequencies for each microsatellite locus in each sample examined. MNR: marine nature reserve. *N*: number of individuals screened/analysed for the locus/sample, –: allele not observed

Locus	Allele	Strangford Lough MNR								
		Cushendall	Tongue	Ballyhenry Island	Saltwater Bridge	Braddock Island	Ballyhornan	Ballyhornan (2006)	Rush	All
Nlw3	178	–	–	–	–	–	–	0.017	0.017	0.004
	180	0.817	0.750	0.617	0.625	0.850	0.667	0.793	0.717	0.730
	182	–	0.100	0.183	0.268	0.150	0.183	0.017	0.067	0.120
	184	–	–	0.017	0.018	–	0.017	0.172	–	0.027
	186	0.183	0.133	0.183	0.089	–	0.117	–	0.200	0.114
	188	–	0.017	–	–	–	0.017	–	–	0.004
	N	30	30	30	28	30	30	29	30	237
Nlw5	162	–	–	–	–	–	0.017	–	–	0.002
	164	–	–	–	–	–	0.017	–	–	0.002
	174	–	–	0.017	0.017	0.050	–	0.016	–	0.012
	176	–	0.117	0.083	0.033	0.033	0.017	0.032	0.017	0.042
	178	0.333	0.367	0.467	0.467	0.483	0.350	0.323	0.050	0.355
	180	–	0.117	0.150	0.050	0.033	0.150	0.145	0.133	0.098
	182	0.033	0.083	0.017	0.050	0.033	0.083	0.032	0.183	0.064
	184	0.483	0.100	0.067	0.100	0.100	0.133	0.016	0.150	0.143
	186	0.017	0.067	0.017	0.067	0.117	0.083	0.161	0.067	0.075
	188	–	0.017	–	–	–	0.017	0.065	0.033	0.017
	190	0.017	0.033	0.050	0.017	0.033	0.067	0.032	0.067	0.039
	192	–	–	–	–	–	0.017	0.097	0.033	0.019
	194	–	–	–	–	–	–	0.016	0.050	0.008
	212	0.017	0.017	–	0.017	–	–	0.016	0.033	0.012
	214	0.017	0.017	–	0.033	–	–	–	0.033	0.012
	216	–	–	0.033	0.050	–	0.017	–	0.033	0.017
	218	0.067	0.067	0.083	0.050	0.017	0.033	0.048	0.067	0.054
	220	0.017	–	0.017	0.050	0.067	–	–	0.050	0.025
	222	–	–	–	–	0.033	–	–	–	0.004
	N	30	30	30	30	30	30	31	30	241

Appendix 1 (continued)

Locus	Allele	Cushen-dall	Tongue	Strangford Lough MNR			Bally-hornan	Ballyhornan (2006)	Rush	All
				Ballyhenry Island	Saltwater Bridge	Braddock Island				
Nlw8	135	–	0.018	–	–	–	–	–	–	0.002
	137	–	–	–	–	0.033	0.017	–	0.017	0.009
	139	0.100	0.018	–	–	–	0.086	0.071	0.150	0.056
	141	0.100	0.018	–	–	–	–	–	0.150	0.036
	143	0.067	–	0.018	0.071	–	0.172	0.268	0.067	0.083
	145	–	0.018	–	–	0.033	0.017	0.018	0.033	0.016
	147	0.017	0.054	0.018	–	–	0.035	0.036	–	0.020
	149	0.083	–	0.071	0.214	0.050	0.190	0.036	–	0.076
	151	0.467	0.679	0.696	0.333	0.650	0.345	0.411	0.100	0.462
	153	0.017	0.143	0.071	0.119	0.017	0.103	0.036	0.083	0.071
	155	–	0.018	0.054	0.167	0.150	–	0.036	0.033	0.054
	157	–	–	0.018	0.048	–	–	–	–	0.007
	161	0.050	0.036	–	–	–	–	–	0.083	0.022
	163	0.100	–	–	–	–	–	0.018	0.083	0.027
	165	–	–	0.018	–	–	–	0.018	0.067	0.013
	167	–	–	0.036	0.048	–	–	0.054	0.017	0.018
	169	–	–	–	–	–	0.035	–	0.017	0.007
	171	–	–	–	–	0.067	–	–	0.083	0.020
	173	–	–	–	–	–	–	–	0.017	0.002
	N	30	28	28	21	30	29	28	30	224
Nlw27	142	0.017	0.052	0.052	–	–	–	–	0.056	0.022
	144	–	–	0.052	–	–	–	–	–	0.006
	146	0.083	–	0.086	0.093	–	0.050	0.016	–	0.041
	148	–	0.241	0.431	0.593	0.283	0.083	0.129	–	0.217
	150	0.017	0.052	0.017	–	–	–	–	–	0.011
	152	–	0.052	0.052	0.111	0.267	0.017	0.032	0.037	0.071
	154	0.883	0.586	0.276	0.204	0.450	0.800	0.774	0.852	0.607
	156	–	0.017	0.035	–	–	0.050	0.048	0.019	0.022
	160	–	–	–	–	–	–	–	0.037	0.004
	N	30	29	29	27	30	30	31	27	233
Nlw11	156	–	–	–	–	0.033	0.050	0.094	0.483	0.083
	158	–	0.017	0.033	–	–	0.017	–	0.083	0.019
	160	0.317	0.733	0.850	1.000	0.617	0.383	0.438	0.017	0.543
	162	0.017	0.067	0.067	–	–	0.017	0.047	–	0.027
	164	0.133	0.033	0.017	–	–	–	0.016	0.017	0.027
	166	–	–	–	–	–	–	–	0.017	0.002
	168	0.533	0.017	0.033	–	0.017	0.033	0.031	–	0.083
	170	–	–	–	–	–	0.033	0.016	–	0.006
	172	–	0.067	–	–	–	–	–	–	0.008
	174	–	–	–	–	–	–	0.031	0.100	0.017
	176	–	0.067	–	–	0.333	0.317	0.109	0.167	0.124
	178	–	–	–	–	–	0.017	0.094	0.117	0.029
	180	–	–	–	–	–	–	0.063	–	0.008
	182	–	–	–	–	–	0.067	0.031	–	0.012
	184	–	–	–	–	–	0.067	0.031	–	0.012
	N	30	30	30	30	30	30	32	30	242
Nlw14	178	–	–	–	0.037	–	–	–	–	0.005
	180	–	–	0.017	–	–	–	–	–	0.002
	182	0.017	0.150	0.017	0.019	0.046	–	–	0.139	0.044
	184	0.817	0.783	0.950	0.907	0.955	0.933	0.952	0.694	0.881
	186	0.100	0.050	0.017	0.037	–	0.017	0.016	–	0.032
	188	–	–	–	–	–	0.050	0.032	0.111	0.021
	190	0.050	–	–	–	–	–	–	–	0.007
	196	0.017	0.017	–	–	–	–	–	0.056	0.009
	N	30	30	30	27	22	30	31	18	218

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