Royal Veterinary College
University of London

Population genetics of the calcifying algae, *Corallina officinalis*

Teresa Vale

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Abstract

Calcifying macroalgae are an integral part of marine communities but they are significantly vulnerable to ocean acidification caused by an increased uptake of CO_2 emissions. Consequently, it is imperative that populations' reactions to ocean acidification be examined by identifying their potential for physiological acclimatisation to environmental changes and/or evolutionary adaptation. Examination of populations across large geographic scales, over which ocean chemistry naturally varies, can reveal "adaptation hotspots" where selection for tolerant genotypes prevails, and thus provide the opportunity to identify mechanisms underlying tolerance. The key to this approach is an understanding of the genetic variation within and between populations across a large geographic range. In the Northeast Atlantic (NE), *Corallina* species are important ecosystem engineers. They are distributed across large latitudinal gradients of ocean chemistry, and have been the focus of recent molecular research that has resolved several species concepts within the genus. In addition, *Corallina* species are model organisms to assess mechanisms of acclimation and adaptation to future change, which require the development of population genetic markers to facilitate such research. This project has used DNA extracted from collections made across the NE Atlantic (England, Scotland, Iceland, and Spain), to identify population genetic markers for *Corallina officinalis* through single nucleotide polymorphisms genotyping. These markers were used to assess population connectivity and genetic diversity for this important calcifying algae over the Northeast Atlantic. An isolation-by-distance pattern was found among this species' populations where Iceland was the most isolated population followed by Spain. However, within the UK fine-scale structuring was also observed, particularly in the South coast with Wembury Point being the most genetically distant population. Regarding climate change, Iceland and Spain may be at risk because they are less genetically diverse and more isolated in relation to the other populations, which might compromise their viability. In contrast, British populations present highly genetically diverse populations which offers them the possibility to adapt to environmental changes.

Contents

1. Introduction

Seaweeds are an important part of marine ecosystems but like many other marine organisms they have been severely affected by climate change (Nelson, 2009). There is growing evidence suggesting that increasing sea surface temperatures (SSTs) and ocean acidification (OA) are the two most significant outcomes of climate change in prompting the adverse effects observed in marine ecosystems (Hughes, 2000; Doney et al., 2012; McCoy et al., 2013). Recent studies demonstrate that oceanic absorption of carbon dioxide and the accompanying alterations in seawater chemistry have unfavourable outcomes for various calcifying organisms which will affect biodiversity, trophic interactions, and other ecosystem processes (Brodie et al., 2014). Therefore, the combination of increasing SSTs and OA carries a serious risk of destruction to marine ecosystems, along with population depletion and elimination (Harley et al., 2006). Consequently, it is urgent that we are capable of predicting and monitoring these alterations in order to fully comprehend the impact that climate change is having on the planet's oceans, and ultimately create mitigation measures for the most susceptible species.

Ocean acidification and increasing ocean temperatures are expected to affect numerous marine organisms. The calcified coralline *Rhodophyta* (red) macroalgae are of particular concern (Brodie et al., 2014). Coralline algae provide a fundamental role to the ecological and biological processes in the habitats where they grow. Work conducted on calcifying reef organisms demonstrated that crustose coralline algae play an important part in the growth and stabilization of carbonate reefs by acting as cement, and contributing towards the consolidation and erosion reduction of coral reefs (Jokiel et al. 2008). Larval recruitment and settlement of various invertebrates are favoured by the presence of these algae which function as ecosystem engineers that provide three-dimensional habitat structure (Adey, 1998; Nelson, 2009). In addition, these algae act as an essential component within the carbon and carbonate cycle and are one of the most substantial biogenic producers of calcium carbonate and primary producers in the costal area (Martin & Gattuso, 2009; Koch et al., 2012; Krause-Jensen & Duarte, 2016).

Although in most benthic coastal areas red calcareous coralline algae (*Corallinales, Rhodophyta)* are abundant and their distribution extensive (Egilsdottir et al., 2012), there is overwhelming evidence that corallines are particularly vulnerable to climate change. Just like with fleshy algae, each calcified alga is adapted to survive within a particular range of temperatures, so it is presumed that their geographic ranges are already changing as a result of global warming, and are predicted to vary substantially as SSTs continue to increase globally (Latham, 2008; Martin & Gattuso, 2009; Brodie et al., 2014). Studies have demonstrated that high temperatures can act synergistically with high pCO_2 to decrease tissue development, as observed with coralline species *Lithophyllum cabiochae* (McCoy & Kamenos, 2015). Moreover, calcified organisms can also be affected by OA through the corrosion of their calcium carbonate skeletons and by increasing the metabolic cost of calcification (Nelson, 2009). These effects of OA are reflected in reduced calcification and consequently low growth rates (Fabry et al., 2008).

However, it has been proposed that individuals currently living in extremely variable settings are probably more resilient to OA. For instance, intertidal species, such as *Corallina* and *Ellisolandia* species, might be more resistant to OA because they can tolerate a wide range of CO_2 levels (Egilsdottir et al. 2012). Their capacity to endure significant pH/pCO_2 variations may be due to adaptation (a common genetic feature within the population) and/or acclimation (a result of the phenotypic flexibility of the individual) (Egilsdottir et al., 2012). Nonetheless, indirect loss of calcified species may still occur via competition with fleshy algal species that benefit from an increase in CO_2 concentrations (Kroeker et al. 2013). Perseverance of species in decalcified forms in environments with greater CO_2 levels can lead to a shift from calcified dominated communities to fleshy algal ones (Johnson et al. 2012). In fact, a substantial decline in calcifying algae within the North Atlantic has been predicted to occur by 2100, which could have tremendous repercussions for local ecosystem stability (Brodie et al., 2014). For this reason, it is essential that population responses to climate change are examined by evaluating their vulnerability to these alterations, along with the prospect for physiological and evolutionary adaptation.

Corallina officinalis represents an important model for investigating the effect of increasing CO_2 on marine calcifying organisms (Kamenos et al., 2013). This is due to several facts, such as: (1) during calcification and photosynthesis they utilise dissolved inorganic carbon (Martin & Gattuso, 2009), therefore influencing CO_2 fluxes within the ocean; (2) they are important for carbon cycling and reef stabilization (Nelson, 2009); (3) there is a wealth of evidence proving their direct reliance on temperature as well as susceptibility to alterations of the sea's chemical composition (Latham, 2008; Nelson, 2009; Brodie et al., 2014); and (4) they can be representative of coralline algae since they have a large geographical range within the North Atlantic. Given the importance of this species to normal ecosystem function (Nelson, 2009), it is vital that we investigate its population genetics so as to understand how populations are connected and which ones are more genetically diverse. Populations with high genetic variability and tolerant genotypes are more likely to adapt to environmental changes (Johannesson et al., 2011). Therefore, the conservation of these "adaptation hotspots" is imperative for the preservation of the species as it permits populations to develop phenotypic plasticity and adaptively evolve in light of future climatic changes (Pauls et al., 2013).

Little genetic research on *Corallina officinalis* exist, and among those published none analyse population genetic patterns (Williamson et al., 2014; Williamson et al. 2015; Williamson et al., 2016). Genetic research for this group has been directed on assessing short sequence regions (genes and barcodes), primarily for phylogenetic evaluation and species delineation (e.g. Williamson et al., 2015). This highlights the rarity of genetic research on corallina algae and red algae in general (Kim et al., 2013). Research utilizing highly variable markers, which are the groundwork for traditional population genetic research, are uncommon for red algae and have been based on microsatellites which are short (1 to 10 nucleotides) sequences of tandem repeats (Kostamo et al., 2011; Song et al., 2012; Vieira et al., 2016). However, single nucleotide

polymorphisms (SNPs) are expected to surpass microsatellites as the most favourable option for population genetic research in the upcoming years (Provan et al., 2013). SNPs have many of the benefits of microsatellites, including codominant inheritance and strength, but they also possess further advantages such as adherence to a simple infinite sites model of mutation, low levels of homoplasy, and possibly thousands of loci accessible for analysis (Provan et al., 2013). Although macroalgae studies involving the usage of SNPs are uncommon and are in their development stages, SNP markers have been established, verified and compared to microsatellite options for the red algae *Chondrus crispus* (Provan et al., 2013). The method utilized (six SNPs) proved to be efficient for the analysis of patterns of intrapopulation genetic variability for this species.

Population genetic studies are essential for the documentation of locally adapted genotypes, and the comprehension of population connectivity is vital for conservation management (Hoffmann & Willi, 2008). Therefore, for this project eleven SNPs were tested on six populations of *Corallina officinalis* from across the UK to evaluate the underlying genetic structure of these populations. In a conservation framework, the information collected in this study can be utilised to identify vulnerable populations of *C. officinalis* or those with local adaptation which can then be applied to develop informed conservation management policies.

2. Methods and Materials

2.1. Sampling and DNA extraction

Samples of *Corallina officinalis* were collected by hand from six locations within the UK: Anglesey, Bembridge (Isle of Wight), Brighton, Firth of Clyde (Scotland), Hebrides and Whitby (Figure 1) – at each locality samples were collected from three different sites (Table 1). Between fifty to forty-nine individuals were sampled from each region, totalling 298 samples. Each sample was given a unique identification and was divided into two sections, one to be dried in silica beads and the other to be pressed on an herbarium sheet, except for the Firth of Clyde population which did not include samples for the herbarium sheet.

For DNA extraction, two methods were used: the modified CTAB microextraction protocol (Robba et al., 2006) and the BioSprint 96 Bio-Robot (Grundmann et al., 2010). Ten out of the 300 samples were processed using the first methodology because they were too small to be processed by the robot. DNA concentration was analysed from samples that were suspended in 300ul of Tris buffer using a nanodrop spectrophotometer to guarantee a satisfactory yield of genomic DNA for genotyping.

Table 1: Summary of the locations within the UK where the populations of *C. officinalis* were collected. N, number of samples collected in each region.

Region	Population	Sites	Coordinates (Lat., Long.)	N

Anglesey	AN	**Site 1:** Bryn Aber	53.406475, -4.517825	50
		Site 2: Hen Borth	53.406602, -4.528673	
		Site 3: East of Carmel Head	53.405182, -4.559862	
Bembridge	BL	**Site 1:** SE of Lifeboat Station, Middle Shore Lagoon	50.688305, -1.069063	50
		Site 2: SE of SITE 1, Higher part of Middle Shore	50.687853, -1.068749	
		Site 3: below SITE 2 in Lower Shore Lagoon	50.688155, -1.068393	
Brighton	BR	**Site 1:** Saltdean	50.799239, -0.038340	49
		Site 2: 500m West of Saltdean	50.801225, -0.048572	
		Site 3: Rottingdean	50.801850, -0.056961	
Firth of Clyde	FC	**Site 1:** 2km South of Meigle	55.839626, -4.891948	50
		Site 2: 500m South of SITE 1	55.836984, -4.891692	
		Site 3: next to Knock Castle	55.827237, -4.889727	
Hebrides	HE	**Site 1:** Little Colonsay, North Harbour	56.455242, -6.262189	49
		Site 2: Little Colonsay	56.447656, -6.262432	
		Site 3: Ulva Bay	56.466924, -6.235313	
Whitby	WH	**Site 1:** Sandsend Ness	54.508889, -0.672704	50
		Site 2: First Bight	54.490724, -0.608681	
		Site 3: Saltwick Nab	54.488254, -0.589468	

2.2. Genotyping

A KASP (Kompetitve Allele Specific PCR) assay was used to perform genotyping (Semagn et al., 2014). DNA samples were diluted in 10 Mm Tris Buffer and retained into three 96 well plates and 10 Eppendorf tubes, at concentrations of over 5ng/ul at 30ul volumes and sent to LGC genomics (www.lgcgenomics.com/genotyping/kasp-genotyping-chemistry) for genotyping. Results were provided as a bi-allelic scoring for each of the eleven SNPs.

2.3. Statistical analysis

The novel SNP data collected in this study was added to data from a previous project (265 samples from Comb Martin, Kent, Iceland, Spain and Wembury Point – see Figure 1) (Jackson, 2016). The statistical analyse was completed by using the statistics software R version 3.1.3 (https://www.cran.r-project.org/). The Linkage Disequilibrium (LD) function from the R package "genetics" (Warnes & Leisch, 2013) was used to compute pairwise LD between genetic markers by utilizing the Bonferroni correction of p-values to evaluate significance. To calculate the observed and estimated heterozygosity (Ho and He) we used the HWE.test function from the R package genetics.

The effect of population structure on genetic differentiation was analysed by using a pairwise.fst function (Weir & Cockerham, 1984) from the R package hierfstat (Goudet & Jombart, 2015). Genetic isolation by distance (IBD) was also tested by conducting a mantel test from the

mantel.randtest function in the "ade4" package in R (Dray & Dufour, 2007), where genetic distances were measured using Fst and geographic distances by using the distance tool in the GIS package Quantum GIS (distance was calculated as distance between locations over water).

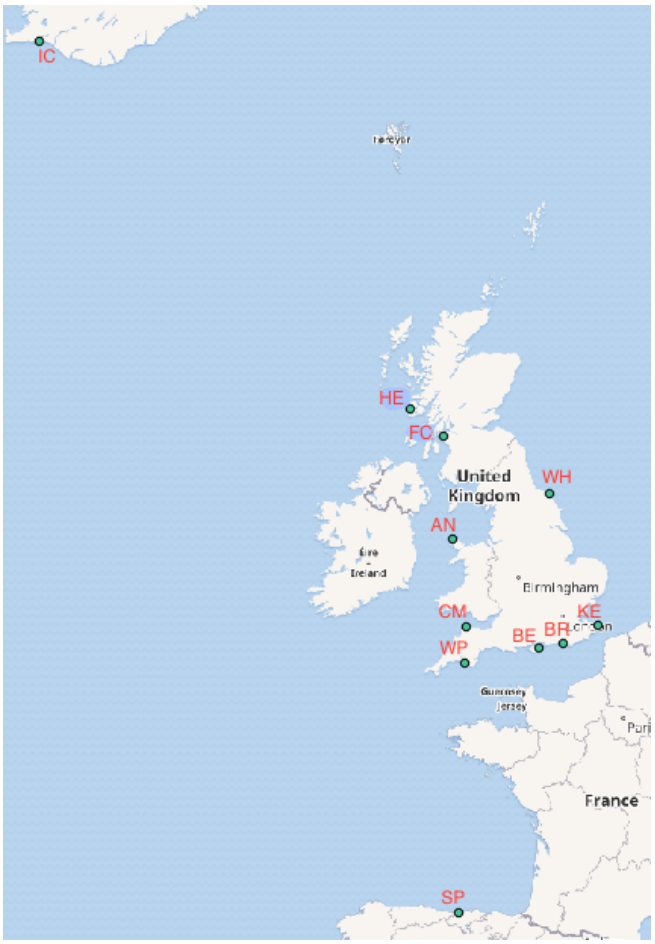


Figure 1: Map of the sample locations for *Corallina officinalis* from current and previous project. Anglesey (AN), Bembridge (BE), Brighton (BR), Firth of Clyde (FC), Hebrides (HE), Whitby (WH), Comb Martin (CM), Kent (KE), Iceland (IC), Spain (SP) and Wembury Point (WP).

To assess genetic patterns and cluster together samples into genetically related groups a Discriminant Analyses of Principle Component (DAPC) was performed using the R package "adegenet" (Jombart et al., 2010). The find.cluster function was utilized to infer the number of genetic clusters, this function calculates successive K-means clustering and evaluates the optimal K by using the Bayesian Information Criterion as reference. The "diffNgroup" (automatic cluster selection) was utilized with a 10^7 n.iter and a 10^4 n.start setting, the selection of principal components to be retained for analysis was based on a percentage of variance threshold (95%). To determine the ideal number of principal components that should be retained the optim.a.score function was used. Lastly, the dapc function was used for the ordination (DAPC) analysis while the compoplot function was utilized to conduct an evaluation of sample allocation to genetic cluster.

3. Results

Two hundred and ninety-eight specimens were sampled and genotyped from six populations. However, results were only obtained for ten of the eleven SNPs. SNP7 was repeated three times with and without optimisation but there was no amplification of samples into clusters, therefore, it failed the quality control checks of the LGC company. All SNPs, except for SNP8 and 10, demonstrated highly significant deviation from the Hardy-Weinberg equilibrium ($p<0.001$; Table 2). Additionally, the LD test revealed substantial linkage among SNPs (Table 3), however excluding the linked markers might have resulted in a dataset that would not

possess sufficient power to produce reliable outcomes, hence, all ten markers were used in the analyses.

Table 2: Candidate SNPs found within *C. officinalis* nuclear genome. Ho, observed heterozygosity; He, expected heterozygosity.

SNP ID	Variation	Ho	He	*p*-value
SNP1	A/T	0.860520	0.808010	1.693e-06
SNP2	G/A	0.787234	0.549430	2.928e-27
SNP3	C/T	0.853428	0.784361	1.551e-08
SNP4	A/T	0.627404	0.509387	9.059e-07
SNP5	C/T	0.726415	0.611250	3.863e-09
SNP6	G/C	0.736077	0.582735	3.038e-13
SNP8	T/A	0.560096	0.520875	0.102
SNP9	G/A	0.646919	0.542477	4.011e-06
SNP10	C/T	0.646635	0.667810	0.238
SNP11	A/T	0.732057	0.512465	1.297e-20

Table 3: Linkage disequilibrium (LD) between the ten SNPs with 5% Bonferroni correction on p-values. TRUE, linkage between SNPs was detected (p<0,05).

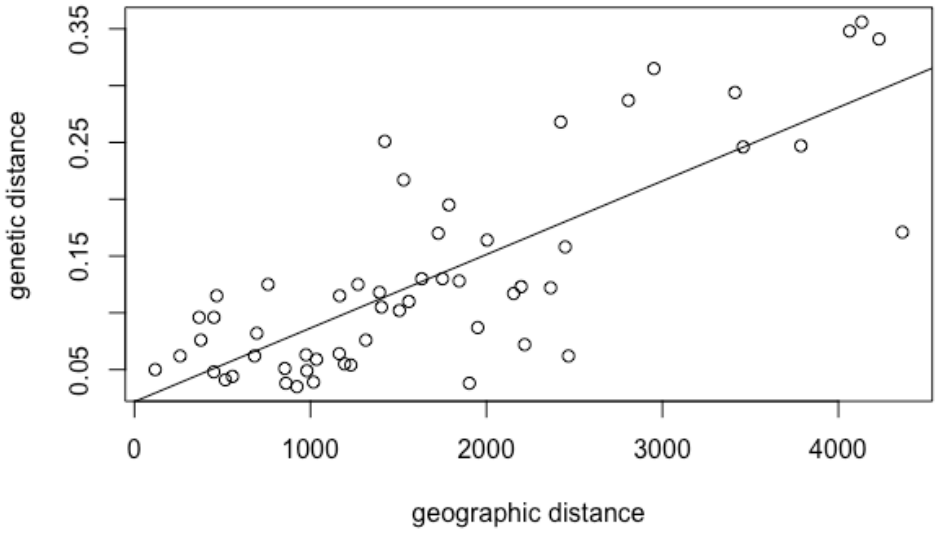
	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP8	SNP9	SNP10	SNP11
SNP1		FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE
SNP2			TRUE	FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
SNP3				FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE
SNP4					FALSE	FALSE	FALSE	FALSE	TRUE	FALSE
SNP5						FALSE	FALSE	FALSE	TRUE	TRUE
SNP6							FALSE	FALSE	FALSE	TRUE
SNP8								TRUE	FALSE	FALSE
SNP9									FALSE	FALSE
SNP10										TRUE
SNP11										

For all SNPs, analysis was performed on samples with at least nine non-null SNPs (N=425, minimum population sample: 21). All regions presented significant genetic differentiation among them (Table 4). The Bembridge and Iceland populations registered the highest genetic distance despite the highest geographic distance being between Iceland and Spain locations (Table 4). Data showed there was meaningful isolation by distance (observed correlation 0.79, *p*=0.001), suggesting that greater genetic divergence is observed in locations that are more geographically distant (Figure 2).

Table 4: Genetic and geographic distances. Upper triangle indicates pairwise Fst values for genetic distances. Lower triangle indicates approximate over-water distances in kilometres. Maximum and minimum values for genetic and geographic distances are highlighted.

Region	AN	BE	BR	CM	FC	HE	IC	KE	SP	WH	WP
AN	-	0.055	0.076	0.041	0.096	0.062	0.315	0.110	0.170	0.072	0.051
BE	1194	-	0.050	0.038	0.130	0.128	**0.356**	0.076	0.251	0.059	0.096
BR	1315	**118**	-	0.049	0.130	0.089	0.341	0.062	0.217	**0.035**	0.115
CM	516	861	980	-	0.063	0.064	0.246	0.054	0.118	0.038	0.044
FC	452	1633	1750	975	-	0.048	0.287	0.164	0.117	0.062	0.125
HE	683	1845	1951	1164	451	-	0.268	0.123	0.122	0.039	0.102
IC	2952	4133	4231	3459	2807	2422	-	0.348	0.171	0.294	0.247
KE	1559	377	259	1230	2004	2197	4064	-	0.196	0.082	0.125
SP	1727	1422	1530	1392	2154	2364	**4364**	1787	-	0.158	0.115
WH	2217	1035	923	1903	2466	1018	3412	695	2448	-	0.105
WP	854	367	467	557	1270	1506	3787	759	1166	1404	-

Figure 2: Isolation-by-distance analyses for *Corallina officinalis* populations in the Northeast Atlantic. The chart demonstrates the geographic distance (km) against genetic distance (Fst) based on ten SNPs.



The cluster analysis elected seven genetic clusters which seem to have a certain level of site-specific partiality. For instance, all Iceland samples except for one form one unique cluster (Cluster 2) and 87% of the Spain samples are grouped in Cluster 4. Nevertheless, there are no clusters that are completely site-specific, for instance there are three individuals from Hebrides, Spain and Whitby that fitted the “Iceland cluster” (Figure 3). The most genetically similar populations are found within the UK where five genetic clusters, which are mainly UK-based and are shared among populations, were formed.

The DAPC utilized seven principal components and six discriminant functions with a proportion of conserved variance of 56% (Figure 3). The scatter plot (Figure 3) demonstrates that the Iceland population (Cluster 2) is more genetically isolated compared to the other populations. Likewise, most specimens from Spain are grouped in a single genetic cluster (Cluster 4), however on the axis the Spanish cluster is more skewed towards UK populations than the

Iceland cluster. Within the UK the most genetically isolated population is Wembury Point followed by the Bembridge population (Figure 3). A close similarity between the Firth of Clyde and Hebrides populations is observed since both populations have a high proportion of samples grouped in the genetic cluster 6 (Figure 4). This similarity can also be seen between Anglesey and Combe Martin populations because both present the same clusters with almost equal proportions for each of them (Figure 4). Cluster assignment probabilities were generally high with 75% of samples assigned with 99% probability (Figure 5). The only Iceland specimen not assigned to the "Iceland cluster" presents a very high assignment probability in favour of the "Spanish cluster" (approximately 90%) (Figure 5). The Spanish population shows a greater affinity towards the UK populations with three samples with an assignment probability of approximately 90% in favour of the "Wembury Point cluster" (Cluster 7). There are a few samples from the Iceland population that show a certain affinity towards the Spanish population and vice-versa demonstrating that even these distant regions have some connection.

Figure 3: Scatter plot visualising the discriminant analysis of principal components (DAPC). Ellipses are centred on each of the 7 genetic clusters. Letters characterize sample sites: A=Anglesey, B=Bembridge, C=Combe Martin, F=Firth of Clyde, H=Hebrides, I=Iceland, K=Kent, P=Wembury Point, R=Brighton, S=Spain, W=Whitby. Proportion of conserved variance is 56%, with axis 1 (x) accounting for 40% and axis 2 (y) 16%.

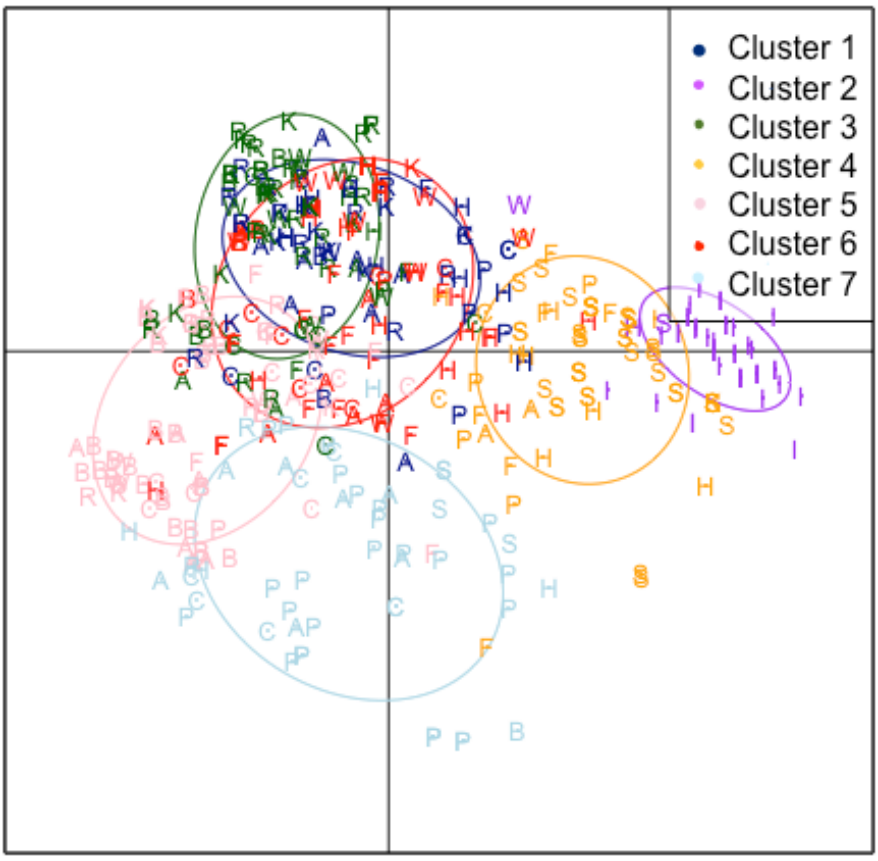


Figure 4: Pie charts representing the spread of the genetic clusters across the 11 populations sampled. Pie graphs indicate the number of individual from each population assigned to each cluster. Colours indicate the assigned cluster(s).

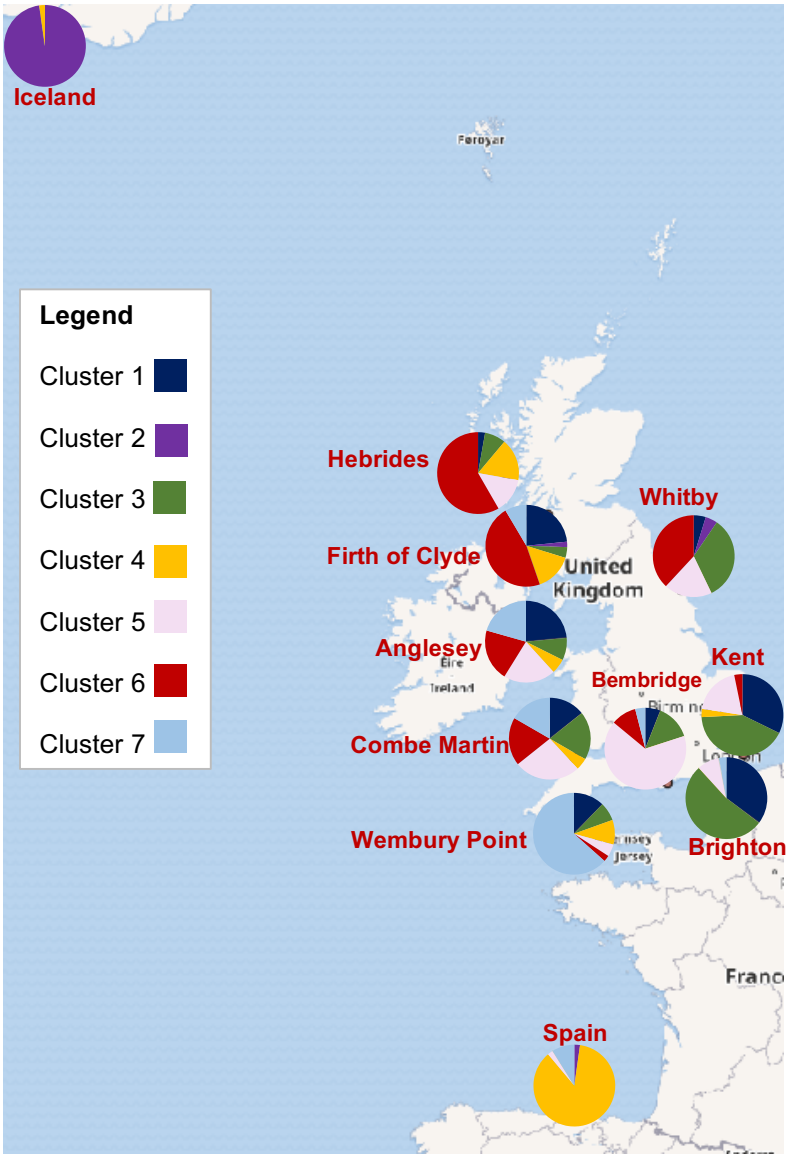
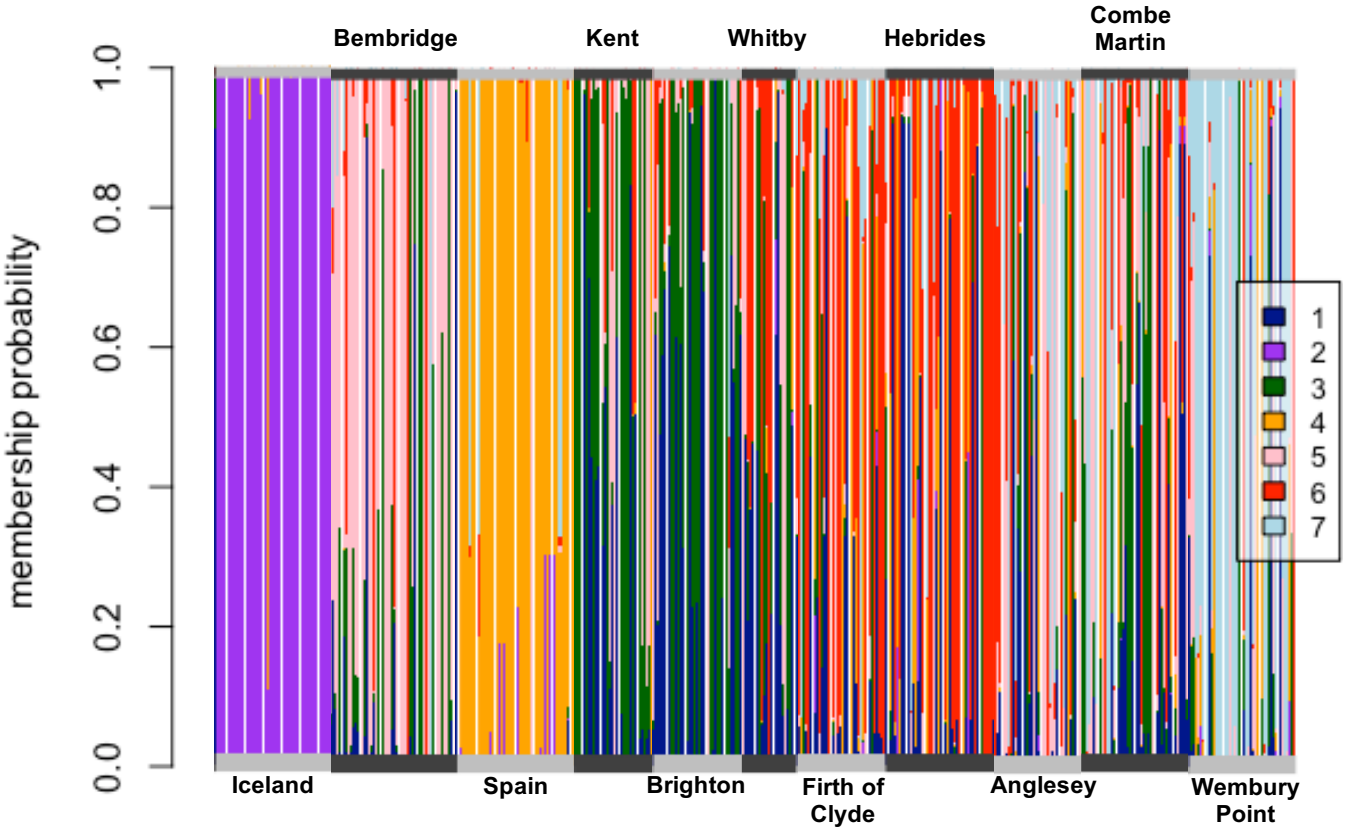


Figure 5: Assignment probabilities of genetic clusters for all individuals. Colours indicate percentage contribution of individuals to assigned clusters (y axis), individuals represented by each line (x axis).



4. Discussion

4.1. Isolation-by-Distance Patterns

Significant results from the pairwise FST test between all populations and confirmation by the DAPC analysis indicate that there is population differentiation in the sampled *Corallina officinalis* populations both at a large- and small-scale. Most of the greatest genetic isolation was detected between the Iceland population and the remaining *C. officinalis* populations (FST= 0.171 – 0.356, 2422 – 4364 km), thus supporting the existence of isolation-by-distance (IBD) patterns among the populations of *C. officinalis* in the Northeast Atlantic. These results are in agreement with findings obtained for other red algae, such as the *Gelidium canariense* species where the IBD model was confirmed (Bouza et al., 2006). The ability of a species to disperse across large distances is often limited by its life-history traits. For example, macroalgae lack “long-lived” propagules as their spores and gametes possess very short survival times which can be shortened by increased water motion (Coyer et al., 2003). Additionally, not even the locomotory abilities of the most powerful algal swimming cells are successful against ocean currents and waves, therefore algal gametes often settle for nearby companions instead of dispersing at larger geographic ranges (Norton, 1992). This is especially problematic for red algae which do not have flagellated cells at all, thereby depending on water circulation for gamete and spores’

dispersal (Hu & Fraser, 2016). Consequently, seaweed dispersal may often depend on overall oceanic water circulation. However, neither of the branches of the North Atlantic Drift, which include the Irminger Current and the Norwegian Current, are successful at linking the Iceland population to the UK or Spanish populations. As a consequence, the reduced aptitude for dispersion of *Corallina officinalis* along with large geographic distances, resulted in limited gene flow between locations which triggered genetic isolation between Iceland, UK and Spain populations. And yet, the isolation revealed here is not absolute as even the most geographically isolated site (Iceland) contained a specimen that presented strong affinity towards the genetic cluster of the most distant location (Spain), more than 4000km away. This may indicate that stochastic mechanisms (e.g., rare events of long distance dispersal) can be important (Zuccarello et al., 2011). For instance, long distance dispersal of gametes and spores may occur when there is fragmentation of fronds bearing reproductive structures or through ship transportation (Valero et al., 2001), thus reducing genetic differentiation between populations.

A pattern of connectivity for *C. officinalis* can clearly be observed along the UK coast where genetic clusters are shared with almost every population, especially in the West coast (from Hebrides to Combe Martin) and East/Southeast coast (from Whitby to Brighton). However, when looking at the South coast an unexpected pattern is detected, as both the Wembury Point and Bembridge Ledges (Isle of Wight) population are less genetically isolated from the Anglesey and Combe Martin populations than they are from each other and Brighton despite geographical distances being greater. Similarly, the species *Chondrus crispus* also demonstrated a tendency for greater differentiation towards the Southern coast of the UK (Hu et al., 2010). These curious patterns could be the result of local oceanic conditions. For example, the English Channel is separated by the Cherbourg peninsula and the Isle of Wight into two main basins: the western and eastern basin. Each basin, presents very distinctive environmental factors and tidal oscillations that are out of phase from one another, therefore the strongest tidal currents occur where the two basins connect (Crisp & Southward, 1958). These currents could be acting as a potential barrier to admixture between Wembury Point, Bembridge and Brighton, thus leading to genetic isolation among these populations. Nonetheless, another factor could also be contributing towards these patterns. Recent studies have proposed that historical factors may play a significant role in moulding genetic diversity patterns across a species' spatial distribution. IBD assessments have been shown to be confounded by historical forces (i.e. environmental changes and/or recent recolonizations) that violate assumptions of equilibrium (Van Der Strate et al., 2003). As a result, IBD models and life-history traits sometimes offer a much simplified explanation that is not always applicable.

4.2. Environmental Change and Conservation

Iceland populations of *C. officinalis* have exhibited potential susceptibility to environmental changes due to high isolation compared to the rest of the sampled Northeast Atlantic populations. With rising environmental pressure, inbreeding may pose a problem as it might

decrease genetic variability even further. Consequently, these populations are possibly susceptible to population decline and might benefit from particular attention regarding conservation related to environmental and climate change. Conversely, the great variability observed in British populations of *C. officinalis* means that these populations might function as a refugium for *C. officinalis*, as perceived in the algae species *C. crispus* in Late Pleistocene (Hu et al., 2010) and *F. serratus* in the Last Glacial Maximum (Coyer et al., 2003). Furthermore, a study on *Corallina elongata* within the UK revealed that periodic fluctuations of pCO_2 could confer population resilience against these changes (Egilsdottir et al., 2012). Hence, British populations of *Corallina offinalis* could become adaptation hotspots against ocean acidification.

Likewise, the great environmental variations that Icelandic populations have experienced during the 20th century could as well result in local population adaptation (Astthorsson et al., 2007; Egilsdottir et al., 2012). Nevertheless, these adaptive mechanisms require a certain level of genetic diversity in order for natural selection to be able to act upon. Additionally, more extreme pCO_2 and temperature fluctuations are expected to occur (Fabry et al., 2008), so the scenario where these populations are adversely affected by OA can not be rejected.

Despite showing residual admixture with the British populations, *C. officinalis* populations from Spain are currently isolated. Therefore, the conservation of this population is vital, especially because of the great macroalgal diversity that is found in this region (Li et al., 2016). However, it was discovered that the Spanish populations of *Fucus serratus* were more resistant to heat stress than UK populations, suggesting that there is thermal acclimatisation in that region (Jueterbock et al., 2014). This adaptation to warmer temperatures might reduce the predicted impacts of global warming on seaweed's distribution ranges. Nonetheless, this resilience has been associated with decreased fitness and reduced reaction to further stressors. So, even though there is a potential for the Spanish populations of *C. officinalis* to adapt to a changing environment, this acclimatisation comes with costs to growth and reproduction which combined with limited dispersal capacity it may still lead to population decline.

4.3. Reservations and Future Research

This research is an important footstep towards the comprehension of the genetic structure of *Corallina*. The SNP markers used in this project show substantial genetic structuring within populations of *Corallina officinalis*, thus confirming some of the findings from the previous study (Jackson, 2016). However, it has been noted that our analyse includes ten markers that present significant linkage. Linkage disequilibrium can cause issues when conducting parental and sibling analysis (Huang et al., 2004), and the presence of linked markers within the analyses could lead to biased results of genetic connectivity among *C. officinalis* populations. On the other hand, if markers with linkage were not utilized, our set of markers might have been to small to analyse. Additionally, the DAPC method that has been used to analyse our data is

designed not to be influenced by linkage, hence it is legitimate to use it on the entire dataset (Jombart et al., 2010).

Another aspect that we need to bear in mind is that only ten SNPs were utilized in this study, which could be considered as a low number of markers when compared to other SNP research where thousands were likely used (Seeb et al., 2011). However, this study demonstrates that meaningful data can be extrapolated from a low number of SNPs, which correlates with what has been observed in the red alga *C. crispus*, where significant genetic structure was detected across the UK using only six SNPs (Provan et al., 2013).

5. Conclusion

Global warming and OA caused by increasing CO_2 emissions are projected to have a severe impact on marine organisms worldwide (Hoegh-Guldberg & Bruno, 2010). Therefore, it is essential that we have a better understanding of populations' genetic structures and on how they are shaped so that extrapolations can be made on the susceptibility of populations to environmental changes and offer predictions on the capacity and speed of evolution for each population. Findings from this study indicate that Iceland and Spain populations of *C. officinalis* require more attention as these populations have shown to be especially isolated and less genetic diverse which makes them more susceptible to population depletion in the context of climate change. On the other hand, the Brittany and Spanish populations may be harbouring more resilient genotypes to climatic changes. These may become important adaptation hotspots for the conservation of the species in the future.

This is one of the few studies that describes SNP markers for a red alga. SNPs are a practical way to examine population connectivity of *C. officinalis* which demonstrated a pattern of isolation-by-distance. This algae species is likely going to be jeopardized by ocean acidification and climate change, but this project can serve as basis for future research.

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