ORIGINAL ARTICLE

Upwelling areas as climate change refugia for the distribution and genetic diversity of a marine macroalga

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ABSTRACT

Aim Global climate change has profound and diverse effects on biological diversity. Identifying present-day climate refugia is an increasingly recognized strategy for the management of biodiversity loss. Such refugia are potential safe havens that enhance environmental diversity by buffering the effects of large scale change, facilitating species persistence at regional scales and conserving unique genetic diversity. Although their ecological effects are well studied, the potential of upwelling centres to act as refugia in a scenario of climate warming remains largely unexplored. Here, we investigate whether upwelled waters act as refugia in a region heavily affected by recent climatic changes.

Location Shores of south-western Iberia and north-western Africa.

Methods We compared changes in distribution of the canopy-forming macroalga *Fucus guiryi* with sea surface temperature patterns in five upwelling areas and adjacent warmer regions. Population diversity and structure was inferred from genotypic analyses using nine microsatellite loci.

Results *Fucus guiryi* has disappeared from large expanses of non-upwelling shores, currently persisting in areas characterized by strong upwelling and reduced or non-significant rates of warming. Populations of *F. guiryi* were more abundant towards upwelling centres. Furthermore, the genetic characterization of populations revealed distinct genetic groups associated with each upwelling system.

Main conclusions Within a large region of predominantly changing climate, we highlight the fundamental importance of upwelling areas as favourable, comparatively stable climates where *F. guiryi* has retreated and persists, preserving unique portions of the species' genetic pool.

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INTRODUCTION

Climatic changes affect biogeographical patterns, causing range shifts and population extinctions (e.g. Nicastro *et al.*, 2013). As a result, the importance of refugia is increasingly recognized (Ashcroft, 2010; Keppel *et al.*, 2012; Gavin *et al.*, 2014). In the past, the term refugium was used to refer to limited spatial locations to which species retreated and where they persisted throughout the Last Glacial Maximum, subsequently expanding to surrounding areas when climatic conditions improved (Ashcroft, 2010; Keppel *et al.*, 2012; Gavin *et al.*, 2014). Several regions have been identified as glacial refugia for both terrestrial (e.g. Beatty & Provan, 2011; Schmitt & Varga, 2012; Meiri *et al.*, 2013) and marine species (reviewed in Maggs *et al.*, 2008). These areas, while often fragmented at present, were responsible for preserving in the past not only species as distinct biological units, but also their pool of genetic diversity (e.g. Neiva *et al.*, 2014, 2015).

Contemporary refugia provide favourable environmental conditions that facilitate species persistence in regions threatened by climate change (Keppel *et al.*, 2012), contributing to the maintenance of regional and global biodiversity. Analogous to past glacial refugia (e.g. Albaina *et al.*, 2012; Meiri *et al.*, 2013), current refugia from warming have great ecological and evolutionary importance and are safe havens that promote biodiversity conservation, allow species to avoid local extinction, and preserve their genetic diversity. Coastal upwelling delivers cold, nutrient-rich water from the ocean's depths to the surface into the intertidal and nearshore region. Depending on the intensity and duration of upwelling events, areas affected by upwelled waters are exposed to temporary or permanent ecological conditions that are distinct from those of surrounding regions. Most importantly, the vigour of coastal upwelling is globally increasing as a result of changes in greenhouse gas concentrations, land–sea pressure differences and wind patterns (Narayan *et al.*, 2010). The delivery of cold waters by upwelling events is not directly correlated with climate, therefore providing an opportunity for regional de-coupling of global warming in refugia, offering insulation from present and projected climatic changes. Although numerous studies have advanced our understanding of the diverse functional roles played by upwelling (e.g. Bosman *et al.*, 1987; Barshis *et al.*, 2011; Rivera *et al.*, 2013), the role of upwelling areas as refugia in a scenario of global warming has been proposed (Riegl & Piller, 2003; but see Chollett *et al.*, 2010), but remains largely unexplored.

The Iberian and north African Atlantic coastlines are particularly suitable regions for investigating the potential sheltering effect of upwelling. Over the last few decades, sea surface temperatures (SST) have significantly increased along this coastline, while five interspersed upwelling areas (west coast of Portugal, Strait of Gibraltar, Cape Ghir, Cape Juby and north of Cape Blanc) show lower or non-significant temperature increase and rather, in some cases, cooling trends (Lima & Wethey, 2012), offering natural experimental replicates. Importantly, these shores are a biogeographical transition region where several warm- and cold-water species reach their northern or southern range limits (Lima *et al.*, 2007; Lourenço *et al.*, 2012; Rubal *et al.*, 2013), therefore offering the opportunity for early identification of distributional expansions and contractions (Lima *et al.*, 2006, 2007; Nicastro *et al.*, 2013; Neiva *et al.*, 2015).

Here, we investigate whether upwelled waters show evidence of acting as refugia in a region heavily affected by recent climatic changes. To do so, we focus on the intertidal canopy-forming brown alga *Fucus guiryi* (Zardi *et al.*, 2011) and compare its recent biogeographical dynamics and population genetic diversity and structure with the geographical distribution of upwelling areas and warming rates over the last three decades.

MATERIAL AND METHODS

Model species

Macroalgae of the genus *Fucus* are a dominant feature along temperate to cold North Atlantic intertidal rocky shores. The recently described species *F. guiryi* (previously designated *F. spiralis* var. *platycarpus* and also referred to as *F. spiralis* Low), can be distinguished from its congeners by the presence of sterile rims around the hermaphroditic receptacles, monopodial branching and the absence of air bladders, in addition to physiological and genetic differences (Billard *et al.*, 2010; Zardi *et al.*, 2011). *Fucus guiryi* is distributed throughout Morocco, the Canary Islands, the Iberian Peninsula and France, northwards to Great Britain and Ireland (reviewed in Zardi *et al.*, 2011). South of northern Portugal, *F. guiryi* is the only *Fucus* species present on intertidal shores (Ladah *et al.*, 2003 – then reported as *F. spiralis*; Zardi *et al.*, 2011, 2015).

Study sites

Fieldwork was conducted along the coastlines of south-west (SW) Iberia, Mediterranean and Atlantic Morocco and Western Sahara, which include three upwelling systems: the Iberian (affecting SW Portuguese shores; Relvas & Barton, 2002), the north-west (NW) African (or Canary, affecting Atlantic Morocco and Western Sahara; Marcello *et al.*, 2011; Benazzouz *et al.*, 2014) and the Strait of Gibraltar (between the Atlantic and Mediterranean shores of Morocco; Stanichny, 2005; see Appendix S1 for detailed description of 'Study sites'). The three upwelling systems comprise five main centres located at: 42°–37° N (PT in Fig. 1, Portuguese coast; Iberian upwelling), 35.8°–35.9° N, 6°–5° W (GB, Strait of Gibraltar); 31°–32° N (GH, north of Cape Ghir; north-west African or Canary upwelling system), 26.5°–28° N (JB, south of Cape Juby; north-west African or Canary upwelling system) and 21°–25° N (BL, north of Cape Blanc; north-west African or Canary upwelling system).

Monthly averaged SST data depicting upwelling seasonality during 2012 with a 4-km resolution were retrieved from the Moderate Resolution Imaging Spectroradiometer-Aqua (MODISAqua) dataset available from the National Aeronautics Space Administration (NASA) Goddard Earth Sciences (GES) Data and Information Services Center (DISC) and used to illustrate the different upwelling centres along the study area (Fig. 1). Visualization was performed using Giovanni, a web-based application developed by the GES DISC.

Distribution and southern range contraction of *Fucus guiryi*

To assess range contraction, the distribution of *F. guiryi* along the Mediterranean and Iberian and African Atlantic coasts was investigated through extensive field surveys on rocky intertidal shores during low spring tides between April

Figure 1 Sea surface temperature (SST) illustrating the five upwelling areas during high (August 2012) and low intensity (February 2012) periods. PT, Iberian upwelling; GB, Strait of Gibraltar; GH, north of Cape Ghir; JB, south of Cape Juby and BL, north of Cape Blanc. Longitude and latitude are represented by horizontal and vertical axes, respectively. Gradient coloration corresponds to sea surface temperature variation. All values below 17 °C are shown in purple, whereas 23 °C or higher temperatures are shown in red.
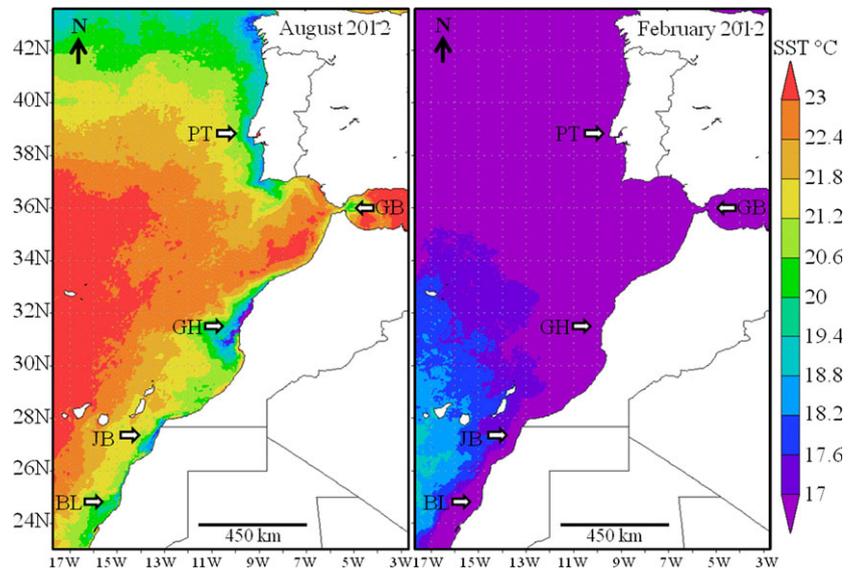
2012 and October 2014 (see Appendix S2). During the surveys, locations where the presence of *F. guiryi* was reported in the past were revisited and additional locations for which no data were available were explored; these locations were selected based on habitat preferences of *F. guiryi* (Zardi *et al.*, 2011, 2015). Thirty-nine locations spreading along > 2600 km of coastline were surveyed. Two observers assessed the presence/absence of *F. guiryi* by performing *c.* 60-min searches across all microhabitats. An additional four non-surveyed locations (Camarinal, Guadalmesi and Punta Carnero; Calaburras R. Bermejo pers. comm.) were retrieved from Bermejo *et al.* (2015), giving a total of 43 study sites.

Historical data on the distribution of *F. guiryi* were assembled from an exhaustive literature review. Literature and information from herbarium collections reporting geographical distribution of *F. spiralis*, *F. spiralis* var. *platycarpus* or mentioning *F. spiralis* Low were considered as reporting *F. guiryi* when populations were described from south of Minho (northern Portugal; see 'Model species'). Published literature was screened up until December 2014 using Google Scholar and the ISI Web of Knowledge with the following keywords individually or in combination: Fucus, spiralis, platycarpus, Morocco, Maroc, Sahara, distribution, Atlantic, Mediterranean, Fucales. AlgaeBase (Guiry & Guiry, 2014) was also consulted and distribution records for *F. spiralis* in northern Africa were considered.

Sea surface temperature rate of change

A subset of 40 points was used to investigate rates of change of coastal SST (warming/cooling data, °C/decade from January 1982 to December 2011) across the study area (see Appendix S1 for detailed description of 'Sea Surface Temperature rate of change'). The selected points were located immediately offshore of each surveyed site with the exception

of two groups of sites that were very close together, for which only one point was available.

Data were analysed by one-way ANOVA in STATISTICA 7.1 (StatSoft Inc., 2005) under the null hypothesis of no difference between sites where *F. guiryi* was or was not recorded. The analyses consisted of presence (two levels, presence/absence) as a fixed factor and rate of SST change as the dependent variable.

Cover occupancy

Visual estimates of cover occupancy of *F. guiryi* along upper intertidal shores were used to investigate the extent of this species at each surveyed site (sites as in Appendix S2; adapted from Bermejo *et al.*, 2015; see Appendix S1 for detailed description of 'Cover occupancy'). The visual inspection was consistently performed by the same observer based on an occupancy scale ranging from 0 (no cover/species is absent) to 5 (massive cover/continuous belts). Cover occupancy from the four non-surveyed locations were retrieved from Bermejo *et al.* (2015).

Genetic data

Samples collected between April 2012 and October 2014 from 13 locations were used to investigate the genetic structure of *F. guiryi* in Morocco and Western Sahara (see Appendix S2). Four additional populations from central and southern Portugal (ER, LZ, AR, CT) analysed by Zardi *et al.* (2015) were included to frame the genetic diversity of this region within the species' overall genetic structure. North of these sites, genetic structure becomes influenced by hybridization with sympatric sister species (Zardi *et al.*, 2011, 2015). Total genomic DNA was extracted using the CTAB method following Hoarau *et al.* (2007). Nine microsatellite loci, L20, L58, L38, L94, L78 (Engel *et al.*,

2003), F26II (Wallace *et al.*, 2004), F9, F42 and F58 (Coyer *et al.*, 2009) were amplified and genotyped as in Perrin *et al.* (2007; for L20, L58, L38, L94, L78) and modified from Wallace *et al.* (2004; for F26II) and Coyer *et al.* (2009; for F9, F42 and F58).

Genetic analyses

Allele sizes were scored using STRAND 2.4.59 software (http://www.vgl.ucdavis.edu/informatics/STRand), binned with the RUBY 1.9.3 package TANDEM 1.09 (Matschiner & Salzburger, 2009) and manually reviewed for ambiguities. MICRO-CHECKER 2.2.3 (van Oosterhout *et al.*, 2004) was used to test for stuttering, null alleles and large allele dropout at each locus and for individuals collected at the same location (hereafter referred to as population).

For each population, observed (H_O) and expected (H_E) heterozygosity and inbreeding coefficient (F_{IS}; Weir & Cockerham, 1984) were estimated, and deviations from Hardy–Weinberg equilibrium were tested for significance with 10,000 permutations. Allelic frequencies were plotted and allelic richness (Â) was estimated and standardized to the two smallest sample sizes. Genetic differentiation between pairs of populations was estimated as F_{ST} (Weir & Cockerham, 1984; and its *P*-values) and as Jost's *D* (Jost, 2008).

To infer population structuring two approaches were implemented: a Bayesian clustering method that uses genetic information to ascertain population membership of individuals without assuming predefined populations (STRUCTURE 2.3.4; Pritchard *et al.*, 2000), and a multivariate analysis (discriminant analysis of principal components, DAPC with ADEGENET 1.4.2; Jombart, 2008 in R 2.15.2; R Development Core Team, 2012).

The presence of hierarchical population structure was also tested (AMOVA; in ARLEQUIN 3.11, Excoffier *et al.*, 2005) which had groups designated a priori based on the Bayesian clustering results (see Appendix S1 for detailed description of 'Genetic analyses').

Lastly, to compare the level of diversity within clusters and within upwelling areas, estimates of H_O and H_E, Â and number of unique alleles were calculated.

RESULTS

Distribution and southern range contraction of *Fucus guiryi*

Field surveys revealed a considerable change in the southern range limit of *F. guiryi* (Fig. 2a). From the 43 studied sites, 24 had past species presence/absence records and 19 were novel. Overall, the species was reported at 23 sites. Eight sites had hosted this macroalga, but no longer did so, although rocky intertidal habitat was available. While only one population disappeared from south-western Iberian shores, the major change in *F. guiryi* range distribution was the loss of all Spanish and Moroccan Mediterranean populations, except

the population at CL. The absence of the species at additional sites not covered by the historical records, along with the study of Bermejo *et al.* (2015) confirmed a marked distributional shift of the species in this region. The northern and southern coasts of Morocco experienced the disappearance of three populations of *F. guiryi*. The coastal locations hosting *F. guiryi* populations were either close to or in the centre of upwelling areas (Fig. 2a).

Sea surface temperature rate of change

All sites showed significant ($P < 0.05$) rate of change of SST except SO, ES and LB, which showed the lowest rates of warming in the entire study area and are located near Cape Ghir and Cape Blanc upwelling centres. In fact, cooling was detected at LB (−0.04 °C/decade), although this was non-significant. The highest warming was in the Mediterranean Sea (PA, 0.32 °C/decade; Fig. 2b). Moreover, sites where *F. guiryi* is absent or has gone extinct showed significantly higher warming rates than sites where the species is currently present ($P < 0.001$).

Cover occupancy

Most of the locations at which the species was present showed negligible or little cover and only one site was characterized by full cover of *F. guiryi* (Fig. 2c). Along south-western Iberia, cover occupancy decreased towards the south from intermediate levels in south-western Portugal at MF to zero at FL. The populations located in the Strait of Gibraltar area generally had little cover occupancy and the remaining Mediterranean population (CL) showed negligible cover. The north-western shores of Morocco showed the highest values and variability of cover. From north to south, cover increased from zero to a maximum at ES (centre of upwelling) and decreased again to negligible cover occupancy values at IM. In southern Morocco and Western Sahara, despite being towards the southern distributional limit for the species, the few isolated locations where *F. guiryi* was present had either intermediate (LB and DK) or large cover occupancy (TF) and were all located inside or near centres of upwelling.

Genetic analyses

The 17 Mediterranean and Atlantic populations included 38 different alleles out of 681 individuals. The total number of alleles per locus ranged from 1 to 18 (see Appendix S3). There was no evidence for large allele dropout at any locus, but potential stuttering and null alleles at a frequency higher than 0.2 were suggested for L20, L94, F26II and F9 by MICRO-CHECKER 2.2.3. All these loci have been used in previous studies (Perrin *et al.*, 2007; Coyer *et al.*, 2011a,b; Zardi *et al.*, 2013, 2015), some of which also suggested potential null alleles (Perrin *et al.*, 2007). However, because the presence of null alleles was not consistent across popula-
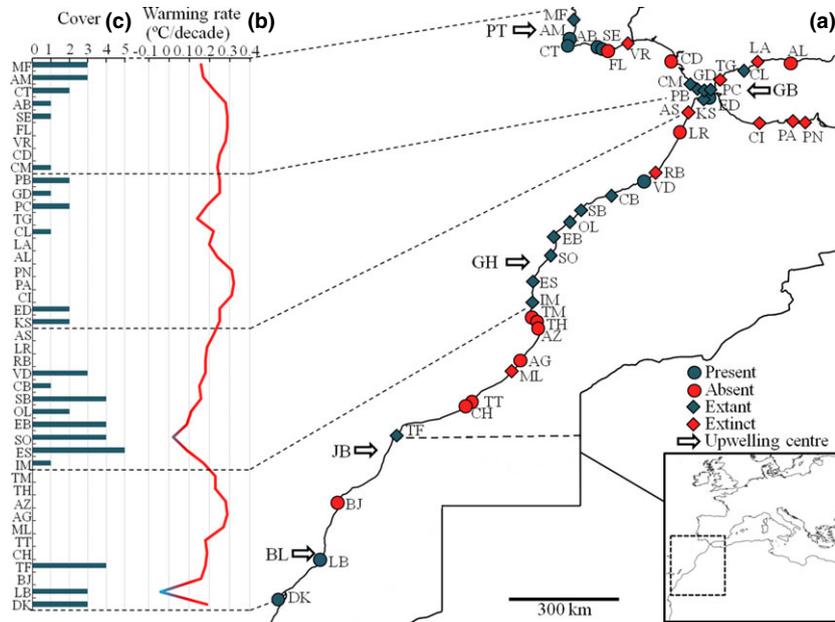
Figure 2 Range distribution of *Fucus guiryi* (a), sea surface temperatures (SST) rate of change (b), and species cover (c) along the study area. Contemporary presence (blue) and absence (red) of *F. guiryi* is based on field surveys (April 2012 to October 2014) and depicted with circles; results compared with published records and herbarium collections are in diamond markers and indicate extinct (red; previously recorded in the literature but absent from present surveys) or extant (blue; recorded in the literature and surveys) populations. Arrows indicate main upwelling centres in the study area: PT, Iberian upwelling; GB, Strait of Gibraltar; GH, north of Cape Ghir; JB, south of Cape Juby and BL, north of Cape Blanc. Coastal SST warming/cooling data covered the last three decades (°C/decade; 1982–2011; http://www.coastalwarming.com/data.html; Lima & Wethey, 2012). Red and blue lines of SST rate of change refer to warming and cooling, respectively. Cover occupancy ranges from 0 to 5.

tions or loci, except for F26II, this may not reflect real null alleles (Perrin *et al.*, 2007). Moreover, the selfing hermaphroditic reproductive strategy of *F. guiryi* (Perrin *et al.*, 2007; then named *F. spiralis*) likely causes an excess of homozygotes, by unequal transmission of different alleles and lack of outcrossing; apparent null alleles are therefore likely. Inbreeding and localized recruitment result in apparent high frequencies of null alleles (e.g. Costantini *et al.*, 2007). These considerations justify the inclusion of all loci in further analyses. Population genetic diversity estimated as allelic richness standardized to the smallest populations $\hat{A}_{(5;\ 22)}$ varied between 1.0 and 1.4 or 1.8, and one population (LB) was monomorphic at all loci. Eleven unique alleles were present over seven locations (Table 1). While ER, ED, VD and DK presented one unique allele each, SB and TF comprised two, and three unique alleles were exclusive to ES. Gene diversity levels estimated as expected heterozygosities (H_E) were always low (0.000–0.189). Observed heterozygosities (H_O) were significantly lower than expected, resulting in a marked heterozygosity deficit ($0.888 < F_{IS} < 1$; $P < 0.001$).

Most of the genetic structure observed between populations and regions was due to differences at loci L20, F26II, F9 and F58. Moreover, locus L78 was fixed in all populations. F_{ST} values ranging from 0.087 to 0.965 were significant ($P < 0.005$) for all pairwise comparisons and supported genetic subdivisions (Table 2). Jost's D ranged from 0.006 to

Table 1 Genetic analyses of *Fucus guiryi* populations along southern Iberia and northern Africa including N, number of individuals per population; H_E, expected heterozygosity; H_O, observed heterozygosity; Â, allelic richness represented by mean number of alleles per locus per population; $\hat{A}_{(n)}$ allelic richness standardized to the smallest sample size; UA, unique alleles; F_{IS}, inbreeding coefficient. Populations codes as in Appendix S2. ER, LZ, AR and CT were retrieved from Zardi *et al.* (2015).

	N	H_E	H_O	Â	$\hat{A}_{(5)}$	$\hat{A}_{(22)}$	UA	F_{IS}
ER	47	0.063	0.007	1.333	1.111	1.267	1	0.888
LZ	48	0.104	0.000	1.556	1.244	1.378	0	1.000
AR	43	0.112	0.005	1.556	1.222	1.467	0	0.955
CT	48	0.047	0.005	1.333	1.000	1.200	0	0.903
PB	5	0.178	0.000	1.444	1.444	–	0	1.000
ED	33	0.148	0.007	1.444	1.311	1.444	1	0.952
KS	48	0.038	0.000	1.222	1.089	1.156	0	1.000
VD	47	0.189	0.000	1.667	1.422	1.644	1	1.000
CB	40	0.039	0.000	1.333	1.111	1.267	0	1.000
SB	37	0.067	0.003	1.444	1.178	1.356	2	0.956
OL	45	0.022	0.000	1.222	1.022	1.200	0	1.000
EB	48	0.043	0.000	1.222	1.089	1.156	0	1.000
ES	47	0.148	0.000	1.889	1.378	1.800	3	1.000
IM	22	0.054	0.000	1.111	1.111	1.111	0	1.000
TF	39	0.189	0.000	1.667	1.444	1.600	2	1.000
LB	48	0.000	0.000	1.000	1.000	1.000	0	1.000
DK	36	0.100	0.000	1.556	1.200	1.489	1	1.000

0.698 and depicted similarly significant ($P < 0.01$) genetic differentiation (Table 2). Bayesian admixture analyses implemented in STRUCTURE 2.3.4 revealed $K = 4$ defined clus-

ters, corresponding to (1) central Portugal (ER, LZ), southern Morocco (TF) and Western Sahara (DK), (2) southern Portugal (AR, CT) and Western Sahara (LB), (3) Strait of Gibraltar (PB, ED, KS) and (4) north-western and western Morocco (VD, CB, SB, OL, EB, ES, IM; Fig. 3a). Three populations (VD, ES and TF) showed high levels of admixture between two clusters (59% and 38% of clusters 4 and 1, respectively for VD; 69% and 22% of clusters 4 and 1 for ES and 52% and 42% of clusters 1 and 4 for TF).

DAPC confirmed the above results, suggesting four distinct groups and highlighting a strong differentiation between Strait of Gibraltar and the remaining clusters (Fig. 3b). The proportion of overall correct assignment of individuals to their prior populations was very high (0.77). The posterior assignment of individuals into each cluster was similar to the STRUCTURE 2.3.4 results. Cluster 1 was widespread along the entire study area, covering central and southern Portugal, Strait of Gibraltar, Morocco and Western Sahara (total of nine locations). Cluster 2 was present in central and south Portugal and Western Sahara. In contrast, clusters 3 and 4 were exclusive to the Strait of Gibraltar or Morocco and Western Sahara, respectively, and were not found outside of these upwelling areas (Fig. 3c). AMOVA attributed most of the genetic variation among clusters (47%; $P < 0.001$), while the other 27% and 26% occurred within populations ($P < 0.001$) and among populations within clusters ($P < 0.001$; Table 3).

Allelic richness by upwelling and by clusters (Table 4) indicated Cape Ghir (GH) and cluster 4 as the most diverse [$\hat{A}_{(39)} = 2.33$ and $\hat{A}_{(36)} = 2.11$] and Cape Blanc (BL) and cluster 2 as the least diverse [$\hat{A}_{(39)} = 1.60$ and $\hat{A}_{(36)} = 1.64$]. Cape Ghir and cluster 4 also had the highest number of unique or private alleles (UA = 9), while either the Portuguese upwelling or the Cape Blanc upwelling showed the lowest (UA = 1) and cluster 2 none (UA = 0).

DISCUSSION

Ongoing change in SST is regarded as the dominant and most pervasive component of climate change, having an impact on species and intra-specific diversity across coastal ecosystems worldwide (e.g. Jones *et al.*, 2010; Nicastro *et al.*, 2013). In this study, we provide evidence that upwelling areas represent contemporary climatic refugia by buffering some populations within a species against ongoing range contraction that is correlated with climate warming. Furthermore, we show that these refugia harbour distinct genetic pools, thereby representing important evolutionary potential for the species as a whole.

Contraction of *Fucus guiryi* distribution into upwelling areas

Sea surface temperature of 71% of the world's shores has increased significantly over the last three decades (Lima & Wethey, 2012). However, this trend is spatially highly heterogeneous, mostly because local and regional phenomena can override and modulate the large scale effect of climate (e.g. Lima & Wethey, 2012). The stretch of coast investigated in our study exemplifies the large scale heterogeneity of coastal SST and warming trends; the general latitudinal thermal cline is interrupted by interspersed areas of upwelling that vary in strength and frequency. Most importantly, SST has significantly increased along most of the stretch of coast in our study (between 0.09 and 0.32 °C/decade for our sampling sites, Lima & Wethey, 2012), but this effect has been strongest in areas distant from upwelling centres.

We show that the intertidal macroalga *F. guiryi* has experienced a recent range contraction of the trailing southern edge of its distribution along extensive stretches of the Atlantic and Mediterranean coasts. However, this large-scale

Table 2 Pairwise F_{ST} (above) and Jost's D (below) comparisons of *Fucus guiryi* populations along southern Iberia and northern Africa. All *P*-values of F_{ST} and D were corrected and significant ($P < 0.005$ and $P < 0.01$, respectively). Populations codes as in Appendix S2.

|	ER	LZ	AR	CT	PB	ED	KS	VD	CB	SB	OL	EB	ES	IM	TF	LB	DK
ER	–	0.087	0.673	0.819	0.788	0.833	0.908	0.525	0.765	0.703	0.804	0.759	0.489	0.725	0.417	0.858	0.349
LZ	0.020	–	0.603	0.741	0.703	0.793	0.870	0.443	0.666	0.600	0.706	0.664	0.398	0.608	0.345	0.743	0.215
AR	0.241	0.242	–	0.440	0.754	0.817	0.887	0.432	0.761	0.719	0.795	0.766	0.600	0.720	0.548	0.796	0.627
CT	0.307	0.290	0.092	–	0.860	0.871	0.934	0.561	0.862	0.822	0.889	0.858	0.707	0.842	0.654	0.891	0.768
PB	0.334	0.348	0.485	0.432	–	0.515	0.633	0.603	0.846	0.787	0.888	0.844	0.622	0.794	0.527	0.953	0.715
ED	0.590	0.589	0.698	0.691	0.282	–	0.599	0.706	0.806	0.786	0.846	0.826	0.712	0.781	0.687	0.891	0.781
KS	0.556	0.556	0.664	0.657	0.148	0.160	–	0.809	0.919	0.906	0.942	0.924	0.824	0.921	0.802	0.965	0.880
VD	0.234	0.219	0.247	0.246	0.442	0.522	0.577	–	0.411	0.336	0.442	0.383	0.257	0.365	0.364	0.659	0.348
CB	0.222	0.222	0.328	0.320	0.370	0.401	0.444	0.151	–	0.488	0.657	0.574	0.390	0.590	0.489	0.920	0.626
SB	0.222	0.222	0.331	0.323	0.402	0.471	0.542	0.140	0.096	–	0.577	0.437	0.224	0.430	0.422	0.863	0.544
OL	0.222	0.222	0.331	0.323	0.403	0.471	0.519	0.141	0.080	0.101	–	0.096	0.470	0.681	0.515	0.950	0.683
EB	0.222	0.222	0.331	0.323	0.406	0.478	0.528	0.125	0.088	0.086	0.006	–	0.351	0.563	0.474	0.902	0.626
ES	0.189	0.188	0.310	0.322	0.386	0.465	0.527	0.120	0.112	0.073	0.127	0.097	–	0.305	0.301	0.688	0.346
IM	0.222	0.222	0.331	0.323	0.406	0.480	0.556	0.173	0.111	0.111	0.111	0.111	0.112	–	0.416	0.918	0.559
TF	0.173	0.176	0.260	0.280	0.339	0.517	0.544	0.232	0.185	0.191	0.169	0.169	0.167	0.194	–	0.682	0.318
LB	0.222	0.204	0.247	0.213	0.407	0.531	0.553	0.264	0.222	0.222	0.219	0.222	0.224	0.222	0.261	–	0.796
DK	0.084	0.066	0.259	0.307	0.399	0.543	0.551	0.149	0.175	0.179	0.179	0.179	0.147	0.179	0.146	0.222	–
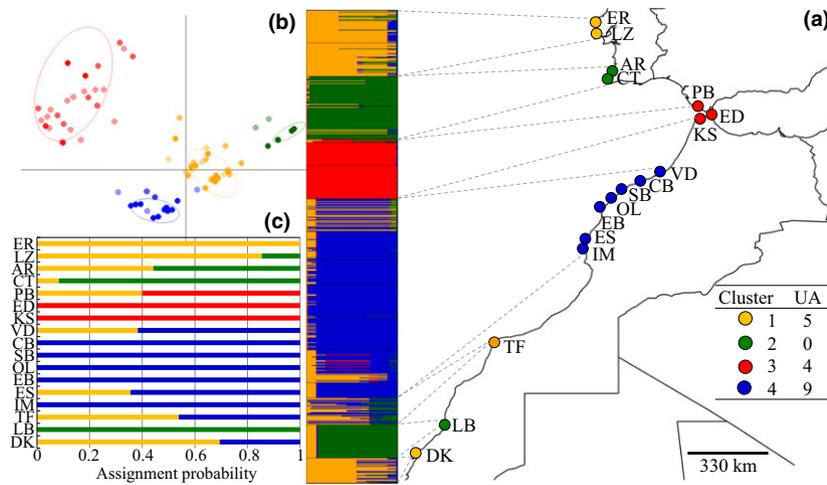
Figure 3 Genetic structure of *Fucus guiryi* populations along southern Iberia and northern Africa. (a) Bayesian analysis summary plot (each bar represents one individual) obtained from STRUCTURE software indicating that clustering of populations is best described by four clusters, i.e. $K = 4$. Solid circles in the map represent the main genetic cluster of each location. UA, unique alleles. (b) DAPC scatter plot of a posteriori $K = 4$ clusters. Each individual was assigned to a cluster and solid dots correspond to overlapping of several individuals. (c) DAPC assignment probability of clusters to each population. In all sections, each colour corresponds to the inferred cluster.

Table 3 Hierarchical analysis of molecular variance (AMOVA) results for *Fucus guiryi* populations from southern Iberia and northern Africa using $K = 4$ clusters as depicted by STRUCTURE. Each population was assigned to a single cluster.

Source of variation	d.f.	Sum of square	Variance components	Percentage of variation	F-statistics	P-value
Among clusters	3	726.589	0.660	47.43	$F_{CT} = 0.474$	< 0.001
Among populations within clusters	13	371.320	0.360	25.86	$F_{SC} = 0.492$	< 0.001
Within populations	1345	499.976	0.372	26.71	$F_{ST} = 0.733$	< 0.001
Total	1361	1597.885	1.392			

shift has not been latitudinally homogeneous from south to north, but rather fragmented. Along the retreating front, extant populations persist within regions affected by upwelling. In contrast, *F. guiryi* is absent or has disappeared from contiguous non-upwelled waters that have experienced significant recent warming. Once abundant and widespread (e.g. Seoane-Camba, 1965; Conde & Seoane, 1982; Margalet *et al.*, 1993; González García & Conde Poyales, 1994), *F. guiryi* has now disappeared from both the European and the African coastlines of the western Mediterranean. Our results show that similar extensive population extinctions of *F. guiryi* associated with recent warming trends have occurred along the Atlantic shores of Iberia and Morocco. The significantly higher SST warming rates in areas where *F. guiryi* is absent or has disappeared compared with locations where it still persists suggest that warming trends underlie local extinction events. In addition to SST warming, several related or unrelated factors may have contributed to the observed range contraction. These include abiotic (e.g. air temperature, waves; Firth *et al.*, 2011; Riera *et al.*, 2015), biotic (e.g. grazing, competition; Walther *et al.*, 2002) or anthropogenic (e.g. pollution, Borowitzka, 1972) factors. However, many studies have

highlighted the role of water temperatures as the main determinant of large-scale range shifts (e.g. Rivadeneira & Fernández, 2005; Jones *et al.*, 2010; Nicastro *et al.*, 2013; Smale & Wernberg, 2013). It is not clear whether seasonal thermal extremes or chronic exposure to stressful thermal conditions caused the range contraction of *F. guiryi*. Extreme climatic events such as marine heat waves can drive marginal populations to extinction (Smale & Wernberg, 2013), but annual mean and maximum water temperatures are also responsible for mortality events in the intertidal zone (Rivadeneira & Fernández, 2005; Jones *et al.*, 2010). Thermal stress can also strongly regulate population dynamics by impairing reproduction (Riera *et al.*, 2015) and algal growth (Short *et al.*, 2015). As populations of an intertidal canopy-forming species suffer a reduction in reproduction and growth, overall abundance is affected (Riera *et al.*, 2015) and algal cover diminishes, in turn decreasing individuals' survival and increasing vulnerability to additional stressors (Brawley & Johnson, 1991).

The absence of detailed information about the past distribution of *F. guiryi* in Western Sahara and southern Morocco hinders our assessment of distributional shifts in this region. Nevertheless, our survey shows that the few known isolated

Table 4 Genetic analyses of *Fucus guiryi* populations from southern Iberia and northern Africa by upwelling and by cluster for $K = 4$ clusters. N, number of individuals per cluster; H_E, expected heterozygosity; H_O, observed heterozygosity; Â, allelic richness; $\hat{A}_{(n)}$ standardized allelic richness to the smallest sample size; UA, unique alleles. Each population was assigned to a single cluster. Upwelling and cluster codes as in Fig. 2 and Fig. 3, respectively.

	N	H_E	H_O	A	$\hat{A}_{(39)}/\hat{A}_{(36)}$	UA
Upwelling						
PT	186	0.1948	0.0042	1.889	1.778	1
GB	86	0.1601	0.0027	2	1.778	4
GH	286	0.1368	0.0004	2.778	2.333	9
JB	39	0.1893	0	1.667	1.667	2
BL	84	0.1272	0	1.778	1.6	1
Cluster						
1	170	0.1503	0.002	2.444	2.089	5
2	139	0.157	0.0032	1.667	1.644	0
3	86	0.1601	0.0027	2	1.844	4
4	286	0.1368	0.0004	2.778	2.111	9

populations (TF, LB and DK) are located within upwelling centres (JB and BL), supporting the idea of the importance of upwelling in mitigating warming effects. Most importantly, populations of this fucoid persist within shores affected by upwelled water regardless of upwelling intensity, frequency or geographical area. Notably, the single extant Mediterranean population (CL) and the few populations still present in southern Portugal (SE and AB) consist of rare scattered specimens (a few tens of individuals in each population) dispersed along the upper intertidal, reflecting a marked decrease compared to the extensive and continuous canopies that prevailed until a few decades ago (Fig. 4 and R. Bermejo, pers. comm.).

Although numerous studies have advanced our understanding of the effects of upwelling on marine systems (e.g. Menge *et al.*, 2004; Nielsen & Navarrete, 2004; Thompson *et al.*, 2012), the refugial potential of upwelling areas in a scenario of global warming has been only hypothesized (Riegl & Piller, 2003 but see Chollett *et al.*, 2010). In this study, we do not aim to investigate which of the distinct components of upwelled waters are more important in providing shelter against ongoing warming. Multiple determinants directly or indirectly related to upwelling may be involved, including SST (Assis *et al.*, 2015), nutrient supply (Pereira *et al.*, 2015) and the moderation of biotic interactions (e.g. grazing and competition, Menge *et al.*, 2004; Nielsen & Navarrete, 2004; Thompson *et al.*, 2012). Despite this limitation, we argue that the assessment of multiple upwelling and non-upwelling areas has provided a strong test for the ecological (and evolutionary, see below) role of upwelling as refugia that extends beyond site specificity.

Interestingly, although other intertidal organisms have shown large scale distributional shifts within the same study area, they did not find refuge within upwelling areas (e.g. Lima *et al.*, 2007; Rubal *et al.*, 2013). For example, the

Figure 4 *Fucus guiryi* cover in 2004 (above) and in 2010 (below) at Santa Eulália (SE in Appendix S2; Fig. 2a), southern Portugal during summer.

southern limit of the congeneric *F. vesiculosus* has shifted over the past 30 years from southern Morocco to central Portugal (Nicastro *et al.*, 2013). Although species distributional modelling identified SST as the most important environmental predictor for the distribution of *F. vesiculosus* (Assis *et al.*, 2014), populations in upwelling areas have also gone extinct. *Fucus vesiculosus* range distribution partially overlaps with that of *F. guiryi*, but the southernmost range limits of the two species are distinct. Presently, *F. vesiculosus*, a cold-temperate water species, occurs from central Portugal (but with few extant, isolated patches in southern Iberia) and North Carolina to the White Sea, Greenland and Canada (Nicastro *et al.*, 2013; Assis *et al.*, 2014), while *F. guiryi*, a southern warm water species, ranges from the Western Sahara to the United Kingdom (Zardi *et al.*, 2011, 2015; this study). These contrasting geographical distributions indicate distinctive temperature optima which ultimately suggest distinct species thermal tolerances and adaptive potential. Taken together with our results, this highlights the fact that the protective effect of upwelling is not applicable to the entire community but it is restricted by species-specific properties of resilience in the face of environmental stressors.

Upwelling areas are reservoirs of genetic diversity

The integration of intra-specific genetic information with distributional data is profoundly important for an understanding of the impact of climate change on species. When genetic diversity of a retreating species is geographically skewed, specific portions of its genetic variability could be under threat, potentially affecting the ability of the species as a whole to adapt to a changing environment and thus increasing its risk of extinction. Here, we identified major genetic discontinuities that are geographically structured along the distributional range of *F. guiryi*. Phylogeographical analyses revealed four distinct genetic groups refuged within the five upwelling centres. This highlights the crucial role played by upwelling areas not only for the persistence of a species as a whole but also for the maintenance of distinct genetic lineages and unique alleles, each nearly endemic to a particular upwelling cell.

Of the four lineages recovered, cluster 4 (Cape Ghir upwelling area) appears to be a relevant genetic resource for the species. The high number of unique alleles and the genetic diversity of clade 4 suggest that this region is a key refugial area for the species with long-term stability, where population persistence and the accumulation of unique mutations are favoured.

In addition, the cluster associated with the Strait of Gibraltar upwelling (cluster 3) revealed the greatest spatial genetic differentiation of all clusters. Given that the species was historically distributed along the Mediterranean coastline, this differentiation, partially caused by the presence of several unique alleles, might reflect an area that was separated from the Atlantic for a prolonged period, causing a distinct lineage to persist in protracted isolation.

Neutral genetic clusters associated with specific environmental regimes may display different physiological tolerances to environmental stress, and thus have unique adaptive potential that can be important for species survival (Pearson *et al.*, 2009; Zardi *et al.*, 2013; Saada, 2014). Potentially, each of the *F. guiryi* lineages described here could display unique eco-physiological responses to their environment, widening the ecological implications of these climatic refugia.

Final remarks

Increased upwelling intensities have been observed over the last few decades (McGregor *et al.*, 2007; Narayan *et al.*, 2010; Cropper *et al.*, 2014) and are predicted to increase further as a response to climatic changes (e.g. Bakun *et al.*, 2010; Cropper *et al.*, 2014; Wang *et al.*, 2015). We can thus anticipate that the contemporary refugial effect reported in this study might even grow, irrespective of the individual nature of each upwelling centre, whether the permanent (100s km) one off Cape Ghir or the small, intermittent one in the Strait of Gibraltar. Concurrently, warming rates are predicted to rise further (Collins *et al.*, 2013) along this stretch of coast,

placing populations at the edges of these upwelling centres under increasing threat.

While the effects of upwelling centres as refugia may be species-specific, in this case there are likely to be bottom-up cascading effects on the ecosystem as a whole. Canopy-forming bioengineer species, such as *F. guiryi*, increase local species richness and diversity, particularly in stressful environments (Watt & Scrosati, 2013). Consequently, the described climatic refugia for *F. guiryi* are likely to indirectly stabilize intertidal species composition, trophic linkages and thus overall ecosystem functioning. In summary, we suggest that upwelling centres are potentially important in maintaining not only the existence of species, which is particularly important in the case of ecological engineers, but also the genetic diversity of species, with long-term evolutionary implications.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Detailed description of material and methods.
Appendix S2 List of sampling locations along Iberian Peninsula and North Africa.
Appendix S3 Allelic diversity at each population and for each locus.

BIOSKETCH

Carla Lourenço is a PhD student at Rhodes University. Her research is focused on the biogeography dynamics and range shifts of intertidal organisms along the upwelling shores of northern Africa.

Author contributions: C.R.L., G.I.Z., E.A.S, C.D.M and K.R.N designed the research; C.R.L., G.I.Z, C.D.M., R.J. and K.R.N. performed the field and laboratory work; C.R.L., G.I.Z., G.P. and K.R.N. analysed the data; and C.R.L., G.I.Z., E.A.S, C.D.M. and K.R.N led the writing.

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