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Evolution of limpet assemblages driven by environmental changes and harvesting in North Iberia

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ABSTRACT: Two limpet species, *Patella depressa* and *P. vulgata*, are the most accessible molluscs in the upper intertidal shore on the coast of Asturias (Northern Spain) and have been consumed by humans for thousands of years, with evidence of harvesting-driven directional selection for size since the Palaeolithic. These 2 species are also highly sensitive to environmental conditions, *P. depressa* being in expansion under the current warming conditions. Our coalescent analyses of mitochondrial DNA suggest that both species are currently in a process of slow decline in population growth after experiencing a recent bottleneck, presumably enhanced by human exploitation. Over a wider temporal scale, the evolution of these species in Asturias seems associated with climate change, which seems to have driven their expansion across the studied zone. Current differential compositions of *Patella* assemblages associated with short-term differences in temperature on North Iberian coasts are consistent with the preferred conditions for each species. Human exploitation may have contributed to the most recent changes in limpet population diversity and morphology (size) at a short temporal scale, whilst climate would have caused long-term changes and would be responsible for most of their present population distribution.

KEY WORDS: Demographic trends · Environmental changes · *Patella depressa* · *Patella vulgate* · Harvesting

INTRODUCTION

Climate is a major factor shaping wild populations (Dawson et al. 2011), e.g. through changes in the geographical distributions of temperature-sensitive species (Thomas et al. 2004), predator–prey relationships (Parmesan 2006), generation time (Gross 2011) and population structuring (Horreo et al. 2011), or through asynchrony of life histories by delayed or advanced migrations (Valiente et al. 2010), through replacement of a species by another with different environmental requirements (Erasmus et al. 2002, Wilson et al. 2007). In intertidal ecosystems, species diversity and composition change along with climate, more pronouncedly in the most recent decades (Helmuth et al. 2006).

Limpets of the genus *Patella* are intertidal species distributed from southern Africa to the Northwest Atlantic and western Indo-Pacific (Christiaens 1973). Ridgway et al. (1998) studied 9 species within this genus: *Patella depressa*, *P. ulyssiponensis*, *P. rustica*, *P. vulgata*, *P. candei*, *P. lugubris*, *P. ferruginea*, *P. caerulea* and *P. pellucida*. Except for the last one, which lives attached to subtidal algae in the subtidal zone, these species live attached to intertidal rocks (Sá-Pinto et al. 2005). Some species have recently expanded their range as a consequence of climate warming, like *P. depressa* (Hawkins et al. 2008) and *P. rustica* (Ribeiro et al. 2010). *P. depressa* exhibits an increasing abundance along English coasts in detriment to other species within limpet assemblages (Hawkins et al. 2008).

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In the Atlantic, *Patella vulgata* prefers boreal to cold-temperate environments, while *P. depressa* prefers subtropical to warm-temperate regions, yet the 2 species coexist from North Wales to the south of Portugal (Fretter & Graham 1976). There seems to be interspecific competition between *P. depressa* and *P. vulgata* in the sympatric area, although stronger intraspecific competition allows their coexistence (Boaventura et al. 2002). If *P. depressa* has recently increased its relative abundance in Britain as a consequence of climate warming, it can be hypothesised that a similar and perhaps more pronounced shift has occurred in the more southern coasts of North Iberia.

Climate, however, is not the only factor affecting limpet species, and other main causes and directions of population modifications should be identified to adequately interpret current species trends. Anthropogenic influences may explain limpet abundance and distribution in the region: these animals have been heavily exploited by humans since the first settlements of hunter-gatherers, mainly for food (e.g. Morales et al. 1998, Gutiérrez-Zugasti 2011). If exploitation is too intense, it can drastically reduce population sizes and induce processes such as loss of genetic variability, changes in population structure and ultimately population decline and resource depression (Myers & Worm 2003, Ward & Myers 2005). Depression of the genus *Patella* after the intense exploitation in the past has already been described (see Mannino & Thomas 2002 and references therein). When several species within an assemblage are exploited simultaneously, as was the case of limpets in the Iberian peninsula (e.g. Morales et al. 1998), substitution of one species by another can occur if one is overexploited, leaving part of its niche free to be occupied by other species (Wing & Wing 2001, Tsahar et al. 2009). An association between the density of Upper Palaeolithic human populations and current limpet size has been detected in North Iberia, together with genetic variation losses in heavily populated areas, which have been interpreted as a signal of old human effect on limpet assemblages (Turrero et al. 2012). Today, limpets are exploited in this region not at a commercial scale but as a low-intensity (small scale) artisanal collection for domestic or local consumption. It is probable that the most intense effects of human harvesting on limpet populations occurred in prehistoric times, although this has not yet been demonstrated.

Given the distribution of the genus *Patella*, its sensitivity to climate change (Mieszkowska et al. 2005, Lima et al. 2006) and its history of past human exploitation in North Iberia, *Patella* assemblages in this region are likely to be good models for examining the relative effect of climate and harvesting on population structuring and genetic diversity. The results can also help assess the current status of the species from this genus in the region and accommodate management regulations to real population biological units and status. Population diversity, structure and demographic trends will be inferred from Bayesian coalescent analyses of cytochrome oxidase I and 16S rDNA mitochondrial sequences, which are of great phylogeographic value for this genus (Sá-Pinto et al. 2005).

MATERIALS AND METHODS

Study sites, temperature data and sampling

This study was conducted in Asturias, a region in Northern Spain (from 43.3944° N, 4.57431° W to 43.5471° N, 7.02304° W) with ~354 km of shoreline, most of it steep and rocky. The climate of the region is temperate Atlantic, with mild winters, moderate summer temperatures and a uniform monthly rainfall distribution (~85 mm mo^{-1}) (AEMET 2005).

Annual sea surface temperatures (SST) were obtained from the Giovanni online data system (http://modis.gsfc.nasa.gov), developed and maintained by the NASA Goddard Earth Sciences Data and Information Services Center (Acker & Leptoukh 2007). We used monthly data from January 2007 to January 2010. The same application can calculate the averages and plots the dates for the study area (Fig. 1).

In Asturias, ongoing artisanal shellfishing is widespread along its coast, without apparent zone differences. We assume the preferential consumption of large individuals. Therefore, differences in exploitation, if any, will be based on size (targeting larger individuals over smaller ones) but not on sampling point or sampled area.

Limpet samples were collected at random from wave-exposed rocks in the upper tidal level of 9 beaches: from west to east, Figueras, Porcía, Luarca, San Pedro, Gijón, La Griega, La Atalaya, Cuevas del Mar and La Franca (Fig. 1). The samples were stored in 100% ethanol and transported to the laboratory for measuring maximum shell diameter and DNA extraction.

Archaeological data sources

Exploited limpet assemblages in different time periods are described by 2 reports on the marine

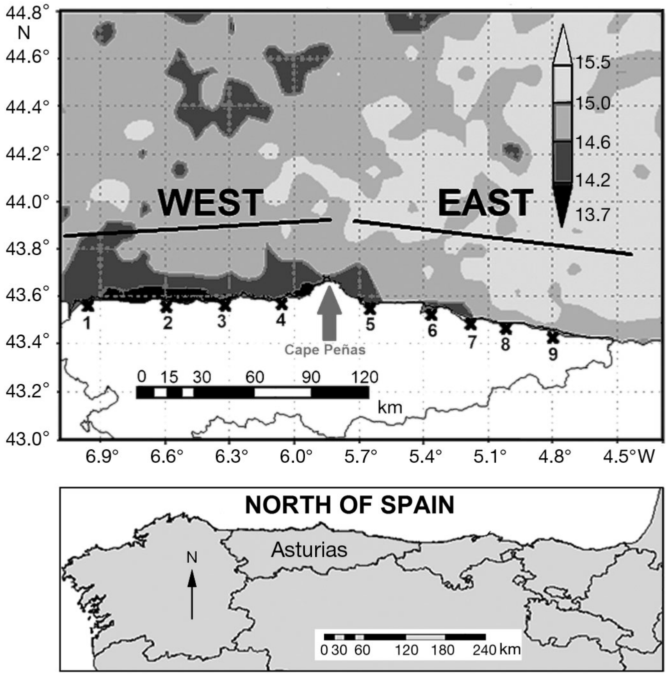

Fig. 1. Sea surface temperature along the coast of Asturias (Northern Spain), averaged from January 2007 to January 2010. ✕ = sampling point (1: Figueras, 2: Porcia, 3: Luarca, 4: San Pedro, 5: Gijón, 6: La Griega, 7: La Atalaya, 8: Cuevas del Mar, 9: La Franca)

faunas of 2 archaeological sites at species level, Poza l'Egua (Arias Cabal et al. 2007) and La Riera (Ortea 1986), located near Cuevas del Mar and La Atalaya, respectively. The 2 sites contain identified limpets dated to the Magdalenian (ca. 16 000 to 12 000 yr before present [BP]), Azilian (ca. 12 000 to 8000 yr BP) and Asturian (ca. 9000 to 6000 yr BP) technological periods. Sample sizes for La Riera were 590, 7648 and 1109 limpet shells from the Magdalenian, Azilian and Asturian periods, respectively, and 25, 51 and 241 for the same periods in Poza l'Egua. The exploited species were reported in the original articles as *Patella vulgata* and *P. intermedia*. The scientific name of *P. intermedia* has been updated here to *P. depressa* following state-of-the-art taxonomic nomenclature for internal consistency.

DNA extraction, amplification, purification and sequencing

DNA extraction was carried out following a resin-based Chelex protocol (Estoup et al. 1996) from a portion of ~2 mm of foot muscle dissected from each specimen. Polymerase chain reaction (PCR) amplification was performed for a fragment of the subunit I of the mitochondrial gene cytochrome c oxidase (COI) and a fragment of the 16S rRNA gene. The primer pairs COI-H-2198 and COI-1490-L were used for COI, with the conditions and reaction protocols described by Folmer et al. (1994); 16S rDNA was amplified using primer pairs 16LRN13398 and 16SRHTB as described by Koufopanou et al. (1999). PCR products were visualized and purified from a 2% agarose gel with a kit by Promega (Wizard SV Gel and PCR Clean-Up System). DNA fragments were sequenced using the ABI Prism BigDye Terminator Cycle sequencing protocol on an ABI Prism 3130 Automated Sequencer at the Unit of Genetic Analysis at the University of Oviedo.

Population structuring analysis

Sequences were visualized and edited using the BioEdit Sequence Alignment Editor software (Hall 1999). The sequences of each gene were aligned with the MUSCLE program included in the MEGA package (Tamura et al. 2011) and were compared with international databases employing the program nBLAST within NCBI (www.ncbi.nlm.nih.gov/). Species assignation was made based on >99% sequence similarity with GenBank voucher specimens of *Patella* species.

The 16S rRNA and COI sequences were concatenated using the DnaSP software version 5.10 (Librado & Rozas 2009) after an incongruence length differences (ILD) test was applied with the program PAUP version 4.0b10 (Swofford 2003) to check if the analysis of the 2 sequences together was congruent. Although this test is controversial for relatively recently evolved taxa (e.g. Yoder et al. 2001), it has been considered to be adequate for phylogenetically old taxa, like molluscs (e.g. Vonnemann et al. 2005).

The program ARLEQUIN version 3.5 (Excoffier et al. 2005) was used to calculate the following diversity parameters: number of haplotypes (Nh, haplotype being each mitochondrial sequence variant), haplotype diversity (Hd, the probability of randomly choosing 2 different haplotypes from the sample) and nucleotide diversity (π, the mean of the nucleotide differences between all pairs of haplotypes in the sample). The same software was used to estimate genetic differentiation between pairs of populations (F_{ST}), measuring the significance of differences in haplotype frequencies between 2 samples, and to perform an analysis of molecular variance (AMOVA) to compare genetic variation between areas within the studied region (i.e. eastern versus western). The latter analysis calculates the percentage of variance accounted for by the differences between groups of samples (for example, between regions), between samples within groups and within samples and gives their statistical significance. ARLEQUIN was also used to check whether there is a relationship between genetic distances (measured by F_{ST}) and geographic distances (in km) with a Mantel test.

Haplotype networks were constructed with the software NETWORK version 4.6.0.0 (www.fluxus-engineering.com). In these networks, different haplotypes are represented by circles connected by lines, which represent the mutations separating them. The diameter of each circle is proportional to the frequency of the corresponding haplotype.

Estimates of demographic trends

We used the concatenated sequences to analyse demographic trends at the species and regional level, separating the coastal areas at the east and west of Cape Peñas (see Fig. 1). The models of nucleotide substitution were obtained separately for each species and gene with the program jModelTest 0.1.1 (Posada 2008) with the Akaike information criterion (AIC). Chronological trends of population growth were retro-estimated employing the program BEAST for Bayesian Monte Carlo Markov Chain analysis of DNA sequences. This software is a framework for testing time-measured phylogenies and performing coalescent analyses; amongst the analyses, it estimates population growth rates across time. We used version 1.6.1 (Drummond & Rambaut 2007), with 0.5% per nucleotide per million years (Myr) as the mutation rate, following Calderon et al. (2008). The results of the BEAST analysis were visualized with the program Tracer v.1.5 (Rambaut & Drummond 2007 and http://tree.bio.ed.ac.uk/software/tracer) to check their stability: the reliability of the estimates was marked as colours (only parameter estimates appearing in black in a run are reliable, i.e. stable, and these were the only ones retained for this study). From the Excel output file provided by the BEAST program, growth rate graphics were constructed with the Microsoft Excel software.

The tree model used in the BEAST program was the Extended Bayesian Skyline Plot. The length of the Monte Carlo Markov Chain was 2.5×10^6 for the western samples of *Patella depressa* and 2×10^8 for the eastern ones (these lengths yielded stable results marked in black by the program Tracer). In the case of *Patella vulgata*, these lengths were 5×10^7 and 6.5×10^6 for eastern and western samples, respectively. In all cases, the first 10% of the chains were discarded as burn-in.

RESULTS

Regional limpet species distribution

Differences in sea surface temperature (SST) are evident in the studied regions west and east of Cape Peñas, being warmer in the east (Fig. 1). A total of 150 limpets (Table 1) were analysed from 4 points located west of Cape Peñas, in the colder part of the studied region (13.7 to 14.6°C), and 187 limpets from 5 points to the east, in the warmer part (14.6 to 15.5°C) (Fig. 1).

The 16S rDNA sequences are available in GenBank under accession numbers JF682557 to JF682592, JF682593 to JF682608 and JF758500 for *Patella depressa*, *P. vulgata* and *P. rustica*, respectively, and were already published by Turrero et al. (2012). The COI sequences for *P. depressa*, *P. vulgata* and *P. rus-*

Table 1. Genetic variability of concatenated sequences of the 16S rRNA and COI genes for *Patella vulgata* and *P. depressa* sampled in 2010 from North Iberia. n: sample size (number of individuals in each population); Nh: number of haplotypes; Hd: haplotype diversity; π: nucleotide diversity. –: low number of individuals or none at all

	n	Nh	Hd ± SD	π ± SD
Patella vulgata				
West				
Figueras	18	8	0.6405 ± 0.1300	0.000802 ± 0.000656
Porcía	36	11	0.4841 ± 0.1041	0.000749 ± 0.000608
Luarca	28	13	0.6825 ± 0.1013	0.001159 ± 0.000834
San Pedro	13	6	0.6410 ± 0.1498	0.000971 ± 0.000767
East				
Gijón	14	9	0.8352 ± 0.1008	0.001803 ± 0.001213
La Griega	3	1	–	–
La Atalaya	1	1	–	–
La Franca	2	2	–	–
Patella depressa				
West				
Figueras	19	11	0.8772 ± 0.0593	0.002823 ± 0.001712
Porcía	1	1	–	–
Luarca	10	7	0.8667 ± 0.1072	0.002375 ± 0.001565
San Pedro	19	14	0.9357 ± 0.0472	0.002500 ± 0.001547
East				
Gijón	24	10	0.7464 ± 0.0907	0.001769 ± 0.001158
La Griega	27	15	0.9174 ± 0.0377	0.002501 ± 0.001522
La Atalaya	43	21	0.8206 ± 0.0582	0.001771 ± 0.001137
Cuevas del Mar	5	4	0.9000 ± 0.1610	0.001993 ± 0.001536
La Franca	38	20	0.8620 ± 0.0528	0.001917 ± 0.001214

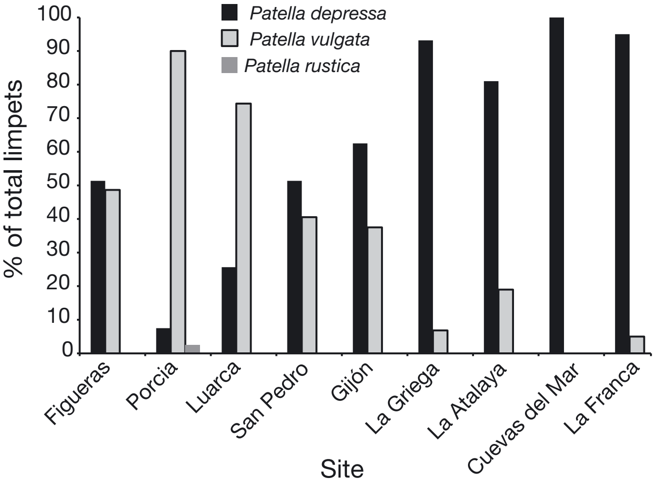

Fig. 2. Percentages of each limpet species at each sampling point (west to east)

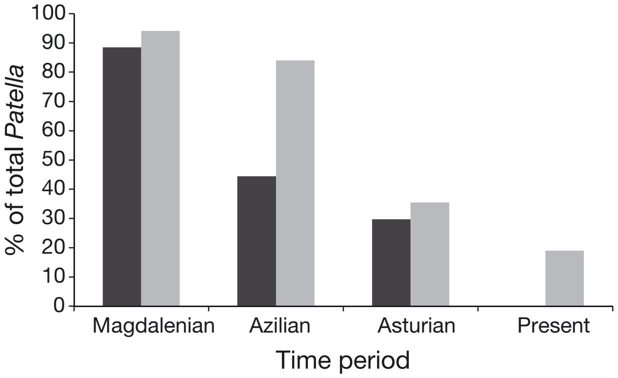

Fig. 3. Percentage of *Patella vulgata* of the total number of *Patella* shells found in 3 Upper Palaeolithic periods (faunal remains from nearby archaeological sites) and in modern samples, in Cuevas del Mar (■) and La Atalaya (□) (North Iberia). Total sample sizes for Cuevas del Mar are 25, 51, 241 and 5 for the Magdalenian, Azilian, Asturian and present periods, respectively, and 590, 7648, 1109 and 43, respectively, for La Atalaya

tica are available in GenBank under accession numbers JF937113 to JF937160, JF937161 to JF937190 and JF937191, respectively. These sequences allowed the unambiguous identification to species level of all of the analysed individuals. The relative proportion of each species was not the same for all sampling sites (Fig. 2). *P. vulgata* was more abundant in western than eastern rocky beaches, whereas *P. depressa* showed the opposite spatial trend, being much more abundant than *P. vulgata* in eastern zones. Only 1 specimen of *P. rustica* appeared, in Porcía (western area).

The archaeofaunal analysis at species level considered in the present study concerns archaeological sites located near Cuevas del Mar and La Atalaya, in the eastern part of the region, where *Patella depressa* is now more abundant. A clear chronological trend is apparent (Fig. 3), wherein the abundance of *P. vulgata* decreased during the studied period in the 2 sites, even down to being absent from Cuevas del Mar today, with the corresponding increase of *P. depressa*. The average size of the archaeological limpets (Table 2) significantly decreased with time ($p << 0.001$ for all *t*-tests between consecutive time periods), and modern individuals of the 2 species were smaller in the east than in the west of the Asturian distribution ($p < 0.05$ for all *t*-tests) (for more details, see Turrero et al. 2012).

Table 2. Maximum shell diameter (mean ± SD, n = sample size) of North Iberian *Patella depressa* and *P. vulgata* west (W) and east (E) of Cape Peñas (archaeological data taken from Turrero et al. 2012)

Time period (yr)	Species		Shell diameter (cm)	n
16 000–12 000 BP	*Patella* sp.		4.16 ± 0.74	615
12 000–8000 BP	*Patella* sp.		3.88 ± 0.75	7699
9000–6000 BP	*Patella* sp.		2.78 ± 0.62	1350
Present	*P. depressa*	W	2.66 ± 0.44	49
		E	2.41 ± 0.70	137
	P. vulgata	W	2.87 ± 0.39	95
		E	2.32 ± 0.48	20

Genetic variation

For the 16S rDNA gene, 53 different haplotypes were found, 36 of which (found in 186 ind.) corresponded to *Patella depressa* (454 base pairs [bp]), 16 (115 ind.) to *P. vulgata* (453 bp) and 1 to the only *P. rustica* specimen (452 bp). Insertions/deletions were not found for any haplotype within the same species. For the COI gene, 79 haplotypes were obtained: 48 for *P. depressa* (650 bp), 30 for *P. vulgata* (656 bp) and 1 for *P. rustica* (670 bp) specimen. Within-species polymorphism in sequence length was not found for this gene.

In the ILD test, the p-values obtained for *Patella depressa* and *P. vulgata* were nonsignificant (0.96 and 0.57, respectively), indicating that the degree of incongruence between 16S rDNA and COI sequences was not very high and that in the present case study, they could be concatenated. The concatenated sequences of the 2 genes were 1104 bp long for *P. depressa* and 1109 bp long for *P. vulgata* and yielded 71 and 42 different haplotypes, respectively. Lower nucleotide diversity was found in the east than in the west of the studied region (Table 1), and the trend was more pronounced for *P. vulgata* than for

P. depressa. The F_{ST} values for each pair of populations were not statistically significant in any case (Table 3). The AMOVA indicated that the main component of the molecular variance was within-sample variation (99.4 % for *P. vulgata* and almost 100 % for *P. depressa*), the regional (between east and west) and among-sample components being not significant. The absence of spatial differentiation was also reflected in an absence of correlation between geographical and genetic distances in the Mantel test (r = −0.153, p = 0.366 and r = 0.002, p = 0.981 for *P. depressa* and *P. vulgata*, respectively).

Demographic trends

Haplotype networks showed 1 more frequent haplotype and a large number of unique haplotypes (carried by only 1 specimen) or haplotypes at low frequencies for the 2 species (Fig. 4). Low-frequency haplotypes were found mainly east of Cape Peñas for *Patella depressa*, while for *P. vulgata*, the opposite trend was found, with more unique haplotypes in the western region.

The model of nucleotide substitution that best fitted *Patella vulgata* sequences (for both genes) was HKY, which gives different frequencies for A-G and C-T changes. The model HKY+I+G best fitted the 2 genes for *P. depressa*; it incorporates a proportion of invariable sites (I) and a rate of variation among sites with a number of rate categories (G; 4 categories in this case). The corresponding model was therefore applied to the concatenated sequences of each species, and the population growth rates exhibited different temporal trends for the 2 species (Fig. 5). In *P. depressa*, maximum growth was first in the west >125 000 yr BP, while in the eastern region, it occurred ~100 000 yr BP. For *P. vulgata*, the situation was the opposite, with a growth rate peaking first in the eastern coast of Asturias >180 000 yr BP and later in the west, ~50 000 yr ago. For both species, the expansive population growth decreased after the marked peak in each region. Since before the Magdalenian period, population growth rates of the 2 species appear to have been decreasing at both sides of Cape Peñas over the years, albeit with a gentle slope (see detailed plots in Fig. 5).

DISCUSSION

Despite relatively small sample sizes in some points, the results presented here allowed us to determine population features of the studied species and to infer the effects of environmental conditions and harvesting. We have primarily found 2 of the 4 species recognized in the region (Borrell et al. 2010), *Patella vulgata* and *P. depressa*. *P. ulyssiponensis* is located at lower tidal levels (Sella et al. 1993), and although *P. rustica* is also found in the upper intertidal, only 1 individual was collected (in the western zone, in accordance with its distribution, supposedly mainly at the westernmost part of the region) (Borrell et al. 2010). No significant population differentiation was found for these limpets, which is consistent with a high dispersal capacity in larval periods (Johnson & Black 1984).

Spatial differences in the relative abundance of the 2 species may reflect different environmental conditions. Cape Peñas represents a temperature boundary within the North Iberian Cantabrian coast, colder to the west than to the east (Fig. 1) due to western upwelling (Dickson & Hughes 1981, Rios et al. 1987). Temperature affects these species in many ways, from determining gonad development (Orton et al. 1956, Bowman & Lewis 1986) to promoting changes in phe-

Table 3. F_{ST} values between pairs of samples as indicators of population genetic differentiation. *Patella depressa* and *P. vulgata* are above and below the diagonal, respectively. No significant values were found. –: low number of individuals or none at all (Cuevas del Mar not shown since this applied to all comparisons for this site)

	Figueras	Porcía	Luarca	San Pedro	Gijón	La Griega	La Atalaya	La Franca
Figueras	—	–	−0.03037	0.00016	−0.00084	−0.01647	0.01773	0.02235
Porcía	−0.00079	—	–	–	–	–	–	–
Luarca	−0.00357	0.0014	—	−0.03404	−0.02428	−0.02637	−0.00005	−0.02328
San Pedro	−0.00674	0.00391	−0.00818	—	0.00799	0.00062	0.02411	0.02193
Gijón	0.00086	0.01857	0.00854	−0.0017	—	−0.00747	−0.01401	−0.01456
La Griega	–	–	–	–	–	—	0.00706	0.00103
La Atalaya	–	–	–	–	–	–	—	−0.00202
La Franca	–	–	–	–	–	–	–	—

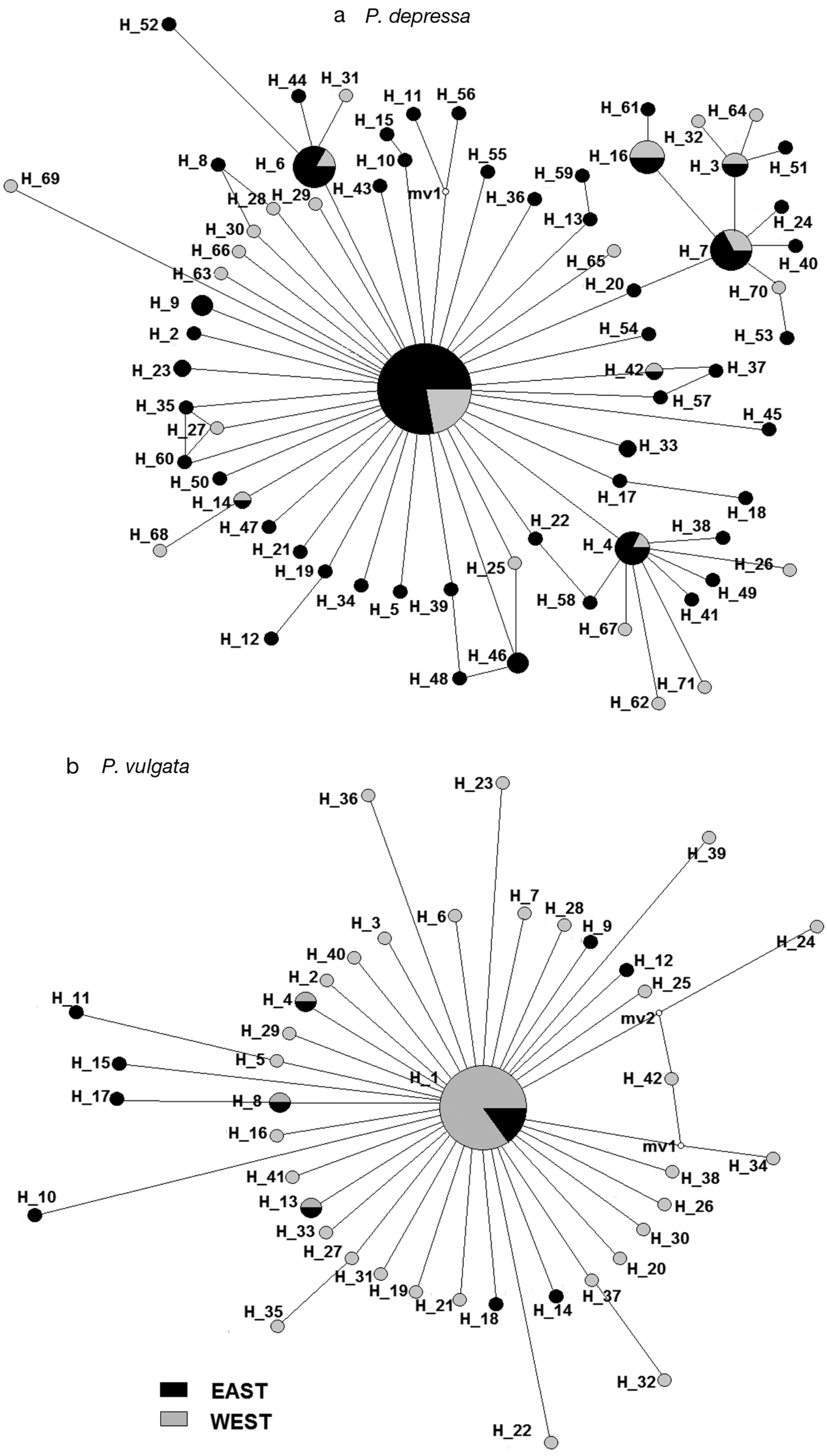

Fig. 4. Haplotype networks for (a) *Patella depressa* and (b) *P. vulgata* from Asturias, North Iberia, based on concatenated mitochondrial DNA sequences (16S DNAr + COI gene; see text). Sampling sites: east and west of Cape Peñas. The hypothetical intermediate haplotypes supposedly existing in the past to explain present variants are shown as white circles

nology (Moore et al. 2011). Low temperatures are a cue for *Patella vulgata* gonadal development; the west of Cape Peñas, with lower temperatures, would therefore be more advantageous for this species and explain its greater abundance in this area.

The long-term demographic evolution of these limpets could be interpreted in terms of climatic fluctuations. For *Patella depressa*, expansive growth would have been initiated in the west (Fig. 5C), in agreement with current limpet assemblages of Portuguese shores, where *P. depressa* is the most common species (Boaventura et al. 2002). According to the BEAST results, such expansion coincided with a time of rising temperatures (~125 000 yr BP) (e.g. de Abreu et al. 2003, Toucanne et al. 2009), which is consistent with the climatic preferences of this species. In contrast, the pattern and timing of demographic expansion inferred for *P. vulgata* would have started at the east (Fig. 5B), with an explosive growth rate increase ~180 000 yr BP. The temperature seems to have dropped during this period (e.g. de Abreu et al. 2003, Toucanne et al. 2009), which is consistent with the preference of this species for colder temperatures. Its expansion in the west of the region would have occurred much later, ~70 000 yr BP, reaching its peak of population growth rate ~50 000 yr ago; this moment also coincided with a descent of temperatures (e.g. de Abreu et al. 2003, Toucanne et al. 2009).

In the last ~10 000 yr BP, rapid climate fluctuations have occurred, with temperatures sometimes above the present average in the NE Atlantic (e.g. Davis et al. 2003). As a consequence, rapid changes in species assemblages of rocky shore ecosystems have been reported, with increases in some species and marked decreases in others (Herbert et al. 2003, 2007, Lima et al. 2006, 2007, Hawkins et al. 2008). Fluctuating environmental

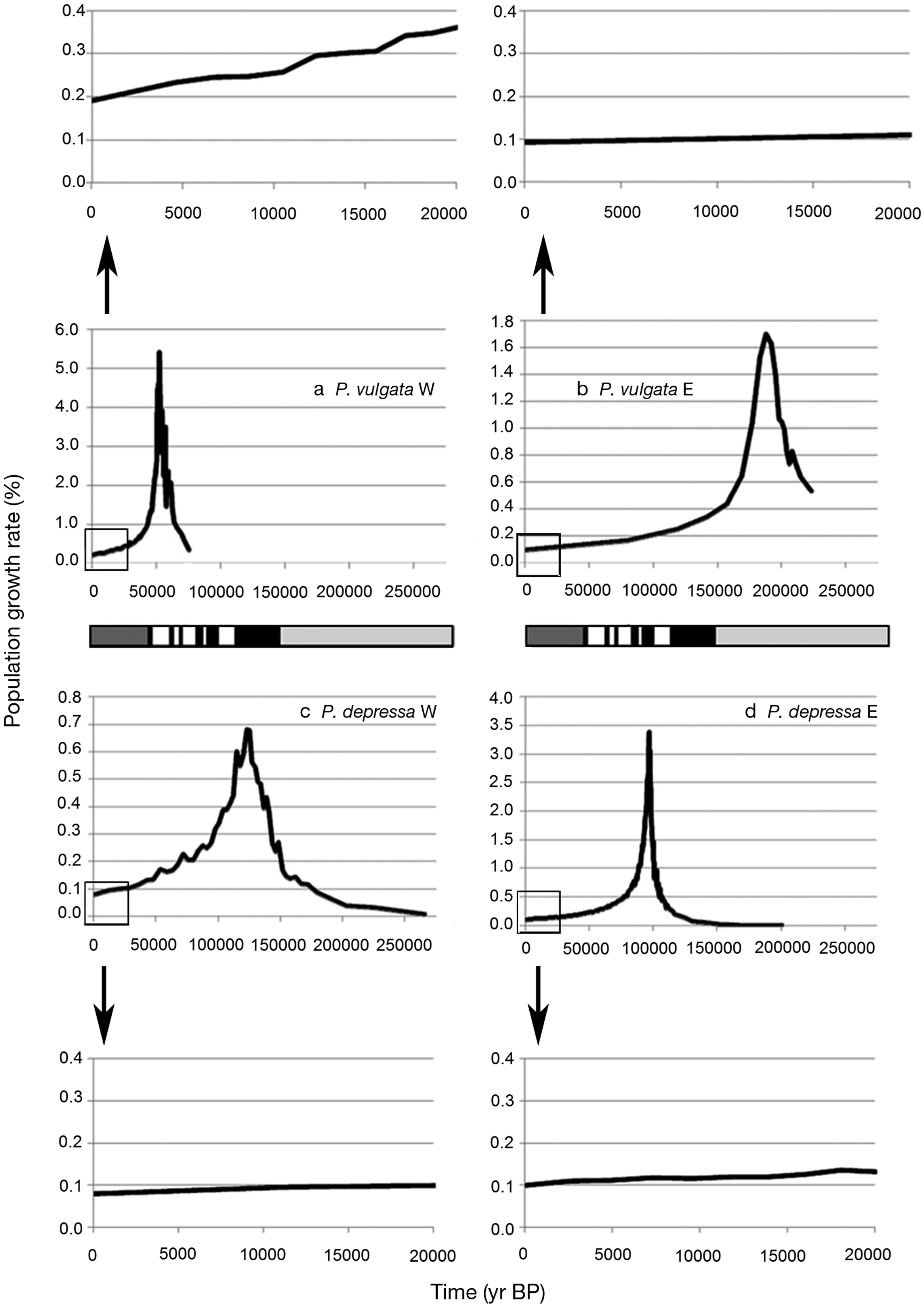

Fig. 5. Population growth rates over time for each species east (E) and west (W) of Cape Peñas (Asturias, Northern Spain). (a) *Patella vulgata* W, (b) *Patella vulgata* E, (c) *Patella depressa* W and (d) *Patella depressa* E. Enlarged plots correspond to the squares in the main graphs and show the growth rate over the last 20 000 yr. Palaeoclimatic data (Pailler & Bard 2002, Sánchez-Goñi et al. 2008) see grey scale: white = cold, black = warm; dark grey = rapid temperature changes; light grey = cold periods with warm peaks

conditions may have adverse effects on the survival of juveniles and larvae as well as on the recruitment to the adult population of the species considered here (Southward et al. 1995). In addition, intraspecific competition is one of the main forces that shape limpet populations. The number of individuals of each species at each point may be determined by intense intraspecific competition rather than by interspecific competition (Boaventura et al. 2002). Intraspecific competition would be enhanced for stressed species under suboptimal environmental conditions, such as higher temperatures in the east and lower temperatures in the west (less preferred by *Patella vulgata* and *P. depressa*, respectively). Demographic growth would consequently decrease and do so more intensely for the more stressed species.

Human populations, in contrast, have increased their consumption of marine species since the Upper Palaeolithic (e.g. Aura et al. 1998, Stiner 2001 Richards et al. 2005, Bicho & Haws 2008). In the past, their impact would have been more pronounced in the eastern part of Asturias, where human settlements were more dense and abundant (Turrero et al. 2012). Traces of past exploitation may be reflected in smaller limpet sizes in eastern zones (Table 2) as a consequence of the selective harvesting of bigger specimens (e.g. Morales et al. 1998, Stiner et al. 1999, Turrero et al. 2012). This likely accelerated the process of species substitution since *Patella vulgata* is larger than *P. depressa* and would be harvested preferentially. However, harvesting is not necessarily the only factor affecting limpet size. Larger size is correlated to lower water temperature in invertebrates, so a concomitant effect of lower temperatures and less intense harvesting might explain the larger sizes in the west. At the population-genetics level, intense exploitation would produce more drastic bottlenecks and diversity reductions in the more exploited species, *P. vulgata* in our study area (e.g. Borrell et al. 2010, Turrero et al. 2012). This would be reflected in its star-shaped haplotype network, indicating past bottlenecks (e.g. Mirol et al. 2008). Therefore, east of Cape Peñas, 2 causes would contribute to *P. vulgata* declines: harvesting and warming. The same 2 causes would be less pronounced to the west, where the species shows higher genetic diversity (Fig. 4). In contrast, *P. depressa* may have expanded along with the warming (Hawkins et al. 2008), contributing to generate the current pattern of relative abundance and genetic diversity of the 2 species at the 2 sides of Cape Peñas. Its past bottlenecks could have occurred during previous cold periods, without excluding past harvesting as in *P. vulgata*.

As seen above, many factors could be invoked to explain the population trends of these 2 limpet species found in North Iberia. As in other invertebrates (Erasmus et al. 2002), climate seems to be a key factor of limpet abundance and distribution (Helmuth et al. 2006, Hawkins et al. 2008). Fluctuating environmental conditions (Southward et al. 1995), intraspecific competition (Boaventura et al. 2002) and human harvesting (Morales et al. 1998, Stiner et al. 1999, Turrero et al. 2012) would also account for sustained declines in these species' demographic growth during the last millennia.

Along the coast of Asturias, *Patella depressa* is more abundant than *P. vulgata* in the warmer eastern area, and this relationship is reverted in the cooler western zone. This distribution is in agreement with the respective optimal temperatures for reproduction of these 2 species. Coalescent analyses of mitochondrial DNA sequences revealed population expansions coincident with climate changes in the last 250 000 yr: warm and cold temperatures would have favoured the expansion of *P. depressa* and *P. vulgata*, respectively. Human exploitation in the last 20 000 yr may have caused differences in size between the eastern and western populations by selective harvesting of larger limpets. Climate seems to have been decisive for population growth at large time scales, while human exploitation would have contributed to produce bottlenecks and morphological changes at a smaller and more recent temporal scale.

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