Localised vs. Regional Adaptation in Limpet Shell Morphology across the Iberian Peninsula

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Abstract: The climate envelope approach to predicting climate-induced species range shift is limited. There are many possible reasons for this, but one novel explanation is that species adapt to changes in temperature at the expense of adaptation to other stressors. Here we test this hypothesis using the limpet, *Patella depressa* (Mollusca, Patellidae), over a large geographical area covering most of the Atlantic coast of Iberian Peninsula, known to consist of a genetically inter-connected population. We examine limpet shell morphology on four shores in each of three regions, from northern Spain to southern Portugal. Within each region, shell morphology (measured as maximum shell profile to length ratio) varied between shore types differing in their insolation, wave action, microhabitat availability and biological factors. However, this ratio, which is known to be an adaptive response to heat stress, was found to be consistently higher in more southern latitudes, despite differences between shore types being found in all regions. This implies that localised adaptation to shore type (most likely through phenotypic plasticity) is compromised by factors which change over latitudinal or regional scales, or which could occur in response to climate change. Even though such climate-induced changes may initially be localized, compromised adaptation (through plasticity or genetic) may result in altered community interactions and potentially large shifts in community structure.

Introduction

Climate change has resulted in the movement of many species' ranges, often towards the poles, but also to cooler higher altitude areas (Walther et al., 2002; Parmesan, 2006; Pecl et al., 2017). The simplest and earliest models of species movement in response to contemporary climate change were the 'climate envelope' models, where species' ranges changed in the same manner as modelled future broad-scale climate variables (Peterson, 2001). However, research has demonstrated that climate related species movement patterns are more complex than this (reviewed by Pearson and Dawson, 2003; Guisan and Thuller, 2005). Small-scale topography can influence climate at a very local scale (Gillingham et al., 2012a; b), and the role of biological factors, including food supply, predation, competition and parasitism, may be important to the final distribution of organisms (Pearson and Dawson, 2003; Guisan and Thuller, 2005; Pecl et al., 2017).

Many organisms are forced far from their physical environmental optima, and this is especially true on rocky shores. Numerous translocation experiments have shown that the flora and fauna inhabiting rocky shores have a lower limit set by competition, and demonstrate higher growth and survival if located lower on the shore in the absence of direct competition (reviewed by Raffaelli and Hawkins, 1996; but see Underwood and Denley, 1984 as exceptions to this rule do occur). 'Stress' in its broadest form, incorporating both physical and biological pressures, is also high on all rocky shores. Theoretical models show the role of physical and biological factors changing over latitude between temperate and tropical regions (Menge and Sutherland, 1974; 1986), but largely imply that increases in biological 'stress' are a result of more benign levels of physical stress.

Fundamentally, these high levels of physical or biological stress suggest that organisms must adapt (through plasticity, acclimation, or evolution) to their localised conditions and major stressors. In some cases, this may mean that organisms will adapt less than optimally to some potential stresses, in order to maximise their response to a major stress (e.g. Stafford et al., 2014).

An example of a morphological characteristic which cannot adapt optimally to multiple conflicting stresses is limpet shell morphology. A high height:length ratio (more pointed shells) has been shown to provide greater ability to cope with heat stress, through increased evaporative cooling (Harley et al., 2009). Response to biological factors, such as competition and predation from crabs and birds, favours flatter shells (low height:length ratio) (Lowell, 1986). Wave action may also play a role in limpet shell morphology. Studies on hydrodynamic lift and drag suggest that forces are lowest when height to aperture radius ratios approach 1:1 (Denny, 2000), and such limpets would have very

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pointed shells, well beyond what is normally found on the shore (Denny & Blanchette, 2000; Denny, 2000). However, flatter limpets would have a higher foot area to volume ratio, allowing them to clamp more firmly to the substratum during wave exposure (Denny, 2000). It is also likely that the initial drag force of the impact of a breaking wave would be lower in a flatter limpet than in a more pointed individual (despite the fact that over the duration of an incoming and outgoing wave, lift would be greater than on a more pointed individual, Denny, 2000). Limpets also respond rapidly to 'threat' conditions by clamping down at the first sign of danger (Coleman et al., 2004), and as such, flatter limpets may be better adapted for survival on wave exposed shores (Cabral, 2007).

Since limpet shell morphology shows differential adaptation to multiple stressors, including heat stress, examining shell morphology along a temperature gradient may allow predictions of how it may change in a given location, over time, with warming temperatures. To examine this idea, this study investigated the role of physical and biological stressors on limpet morphology over a latitudinal and temperature gradient. Shell morphology of *Patella depressa* was examined between northern Spain and southern Portugal over a range of rocky shores, which differ in the biological communities present, wave exposure and aspect (and hence thermal stress). We hypothesised that moving southwards will increase the general levels of thermal stress, and that limpet shell morphology has adapted (either through phenotypic plasticity or evolution) to create more pointed limpets in response to higher heat stress, regardless of the other localised pressures.

Methods

Four different sites were examined in each of three regions in northern Spain, western Spain and southern Portugal (Figure 1), during late June and early July 2014. These regions fall into different temperature categories with mean summer air temperatures of 17.5, 20.0 and 22.5 ˚C respectively (AEMET-IM, 2011; Figure 1). Despite these differences in temperatures, the geographic range studied was predicted to show high levels of genetic interconnectedness for *Patella depressa* through planktonic dispersal, with potential limits for gene flow immediately to the north and south of the studied range (Ribeiro 2008). Within each region, two isolated sections of rocky shore, which were separated by > 100m but < 500m, were examined on each of two beaches. The beaches themselves were between 2 and 8 km from each other. The sections of rocky shore on each beach were chosen to differ in either wave action or aspect, hence insolation. Given limited scope to move

between sections of shore and the homing of *Patella depressa* in these regions (Silva et al., 2003), aspect of the shore should be a good indication of insolation the limpet received during post-settlement development, and other than changes in insolation due to microhabitat selection, the shore sections were similar in aspect and exposure throughout their length. The beaches themselves were chosen in terms of proximity to each other and accessibility, and hence were chosen for reasons of practicality rather than for any specific features.

For each shore, the height (above chart datum) of maximum limpet density (*Patella depressa*) was established by a brief 5 min search. This height was not a constant between shores and was recorded for subsequent analysis. The difference in shore height between wave exposed and non wave exposed shores varied by as much as 2.8 m. Hence, choosing a fixed shore height to conduct studies with different levels of wave exposure is not practical. As described in the data analysis sections, changes in shore height form part of the shore classification process. Measurement and accounting for these changes is therefore what we believe to be the best approach. A 20 m transect was run along the shore at this tidal height and 30 *P. depressa* were selected randomly (as the closest individual to the random coordinates) for measurement. The maximum shell length of each limpet was measured to the nearest millimetre using callipers. To avoid removing excessive numbers of limpets from rocks, the profile of each limpet was measured by placing string along the shell over the longest axis and measuring its length. This method was validated against 10 limpets which were removed from the rock and photographed for digital analysis; there was a difference of < 1 mm between the methods. To ensure consistency of measurement, the same person recorded the shell lengths and profile lengths of each limpet throughout the study. Periodically (every 10 limpets) checks were made by re-measuring the limpets a second time. To ensure that all limpets measured were *P. depressa*, rather than other species, limpets that could not be easily identified from their shells were removed from the rock for further investigation, including foot colouration. Typically this involved only one or two limpets per transect, as the morphological differences between species on each transect were easily identified.

To determine the characteristics of each shore 20, 0.5 x 0.5 m non-overlapping quadrats were characterised along the transect, resulting in 50% of the transect being sampled. In each quadrat, the total number of each species of limpet, other grazers and limpet predators (e.g. dogwhelks) were counted. Predators were later excluded from the analysis due to very low numbers present at all sites. Percentage coverage of barnacles and mussels were estimated and the percentage coverage of crevices (large enough to hold at least one typical limpet), and standing water (rock pools) were also recorded. The complexity of the shore topography was also calculated by

establishing the distance along the rock surface of the 0.5 m displacement of the top and left edge of
130   each quadrat, and this measure allows for an understanding of variation of insolation and exposure
along the shore caused by microhabitats to be present in the shore classification process.
Aggregation of limpets was measured as this is considered a possible behavioural mitigation against
predation (Coleman et al., 2004). To assess this, five limpets were randomly chosen in each quadrat
and the distance to their nearest neighbour measured. In cases where the shells touched the
135   distance was recorded as 0 mm. Limpets were chosen independently, so where a reciprocal nearest
neighbour was found (i.e. a chosen limpet had previously been a nearest neighbour limpet), this
individual was ignored and a new limpet chosen. Where fewer than five independent limpets could
be found in the selected quadrat, limpets in quadrats immediately above and below were also
measured.

140   The GPS coordinates of each shore were recorded to determine temperature and exposure stresses
using the methods of Stafford et al. (2014). Sites were scored on an ordinal scale for exposure to sun
(north facing = 1, east = 2, west = 3, south = 4) and wave exposure (0-4 scale). Wave exposure was
based on predominant swell direction for each site, obtained from seasonal data available from
MetCentral Ltd and from analysis of fetch from the sites (i.e. shelter from headlands or near shore
145   islands reduced the exposure of some beaches). For example, a shore facing directly into the
direction of the predominant swell direction in the region, and with no headlands of shelter to
deflect swell would score a 4 for wave exposure. However, a shore facing directly away from the
predominant swell direction would receive a 1 for wave exposure, or zero if it also was sheltered by
headlands.

150   Details of solar and wave index, along with density of *P. depressa,* density of other grazers,
percentage cover of mussels and barnacles, percentage cover of 'geological' shelter (consisting of
crevices and rockpools), the average nearest neighbour distance, shore complexity and tidal height
of the transect (and hence the highest abundance of *P. depressa*) were converted into percentage
components of categorisation of the shore (as per Stafford et al. 2014). The concept of this shore
155   classification is that stress on any given shore can be mainly physical (e.g. temperature or wave
action) or biological (e.g. competition or predation) or a combination of both, and that by expressing
all variables as a percentage of their maximum (defined as the maximum on any shore studied in the
survey) as well as ensuring that overall each shore has an equal amount of stress (regardless of the
nature of this stress) then the shores can be compared.

160   To achieve this percentage weighting; 1) all measured variables at a given site were standardised by
dividing by the mean value for that variable across all sites. 2) The percentage value of each factor at

each site was then calculated by dividing the standardised values, obtained in step 1, of each factor at each site by the sum of all factors for each site and then multiplying by 100 (Stafford et al., 2014 provides step by step guidelines for this process). Ultimately, this meant that a shore with a high wave index, but low sun index, grazer density and complexity would be dominated by the wave action score, and this would be considered the major stress acting on the shore. A shore with low solar and wave indices, but high levels of grazing would be dominated by competition stress. Other factors such as complexity could be considered mitigation against solar or wave stress at a microhabitat level, so were included in the categorisation of stress on the shore. Equally, shore height was included in the shore classification as it was not possible to standardise for this across the levels of exposure investigated (see above), and if shore height was important in distinguishing between shores and affecting the shell morphology of limpets, it could be seen in way in which the shores were classified into groups.

Percentage weightings for each shore were then applied to a k-means classification algorithm. Three groups were chosen for classification following the use of the 'elbow' method of determining number of groupings (Thorndike, 1953). The ratio of limpet profile over length (herein referred to as pointedness) was used as a dependent variable in a two-way ANOVA, where the site classification and location (N. Spain, W. Spain, S. Portugal) were both fixed factors with three levels each in the analysis. Since pointedness ratios were typically > 1.2 no transformation of the dependent variable was necessary (i.e. data were not close to the artificial floor created at 1). To further examine the effect of wave and solar stress with latitude, a sun/wave index was calculated by dividing the sun index by the wave index, then $\log_4$ transforming (the untransformed data were negative skewed and the $\log_4$ transformation normalised the distribution, given that most values were distributed between 0.5 and 4, given the scales these values were measured on initially). An ANCOVA was conducted using the transformed sun/wave ratio as a continuous variable and the location as a fixed factor as described above. The interaction term was also included in the ANCOVA.

Results

The relative importance of different stresses or stress-reducing factors on each shore differed greatly between shores (Table 1). Following the k-means classification, shores with classification 2 were generally dominated by wave action. While not so clear, those with classification 1 showed higher sun/wave ratios (although this ratio was not a classification factor in the k-means classification), and those with classification 3 showed higher levels of geological refuges (rock pools and crevices) (Table 1).

The pointedness of limpets varied significantly between site classifications as designated by the k-means algorithm ($F_{2,352}$ = 11.5; $p < 0.001$; Tukey test 1 = 2 > 3 at p=0.05 significance), and between the different locations ($F_{2,352}$ = 5.70; p = 0.003; Tukey test Portugal > W. Spain = N. Spain at p=0.05 significance). However, there was no significant interaction between these factors ($F_{3,352}$ = 1.19; p = 0.315). It was clear that pointedness of limpets increased from the Spanish sites to the Portuguese sites, and this change could be seen across all site classifications (Figure 2; note there were no sites of type 2 in W. Spain).

The sun to wave index also showed more pointed limpets at shores with higher sun or temperature stress ($F_{1,354}$ = 7.42; p = 0.0075; Figure 3). There were again significant differences between the regions, with Portuguese limpets showing higher pointedness indices than the other regions ($F_{2,354}$ = 4.55; p = 0.011; Figure 3). Again, no significant interaction term was found ($F_{2,354}$ = 0.107; p = 0.90).

Discussion

Limpet shell morphology differs between different shore types in a consistent manner between regions, indicating different stresses such as temperature or wave action affect the shape of their shells. However, moving from more northerly (cooler) regions to more southerly (warmer) regions, there is also a consistent trend for more pointed shells in the limpets. Pointed shells are an adaptation to temperature (Harley et al., 2009), which occur over a temperature/latitudinal gradient despite alternative stresses such as wave action also being extremely high at some of the southerly shores. Equally, an alternative analysis of the data demonstrated that shores with high wave exposure had flatter shelled limpets compared to those with higher levels of isolation. Again, this trend occurred across all three regions, but limpets in the southern most region (Portugal) were more pointed across all combinations of wave and sun conditions.

From measuring a wide range of physical and biological characteristics of the shores studied, three categories of shore were identified by our statistical methods. These shore classifications could not fully be assigned to the hotter, south or west, aspect shores or to higher levels of wave exposure, but there was an indication that one category was at least partially defined by increased wave action, one by a higher amount of insolation as compared to wave action, and one by increased levels of geographic topography such as crevices (a stress mitigation feature). Nevertheless, these differences between shore classification created consistent differences in limpet shell morphology across the different regions studied. Given limpet shell morphology is highly variable (Moore, 1934; Denny, 2000; Harley et al., 2009), but often non-optimal for any single given purpose (Denny 2000), these differences in shell morphology found across shore classifications likely occurred as a result of

an optimal adaptation to the stresses on the shores as a whole (Chevin et al., 2010), even if some of these stresses can not be fully quantified by the current study.

Across the latitudinal gradient studied (~ 5 degrees), however, there was a tendency for limpets to be more pointed at lower latitudes. While consistent differences occurred between shore types in all three of the geographic regions studied, limpets in the hotter, southern-most region were always more pointed than those found further north (see Vermeij, 1973; Harley et al., 2009 for similar results relating to morphology with temperature change in intertidal species). Again, these changes in morphology are likely to be related to optimal adaptation to the shore. However, it raises the important concept that on shore classification 2 or 3, where limpets were found to be less pointed than on shore type 1, (or on shores with lower sun:wave ratios) this increase in pointedness at lower latitudes must be related to inferior adaptation to whatever stressors cause the limpet to be flatter at higher latitudes (e.g. wave exposure, competition or predation: Lowell, 1986; Cabral, 2007). Hence adaptation to temperature is occurring at the expense of other adaptations to local conditions.

While it is not absolutely clear from this study whether changes in shell morphology are due to phenotypic plasticity or evolution. Clearly some degree of plasticity occurs, as sites within each of the three studied regions (separated by 100s metres to several kilometres) differ in morphology and limpet shell morphology has been found to change in transplantation experiments with shore height (Moore 1934). Studies on the dispersal of *Patella depressa* across Spain and Portugal suggested that connectivity through planktonic spawning was high in the area studied (Ribeiro, 2008). As such, changes in morphology are likely to be due to plasticity to the local conditions, rather than genetic adaptations.

The shell morphology measurements obtained in this study over the latitudinal gradient are a potential indication of compromise to multiple stressors with conflicting demands. In this case, changes in morphology occur both to localised stressors (aspect, other biota and wave action, for example) and the latitudinal gradient studied. As such, limpets optimally adapted to wave exposed local conditions in northern Spain will be less optimally adapted to local wave exposed conditions requiring flatter shells if they were translocated to southern Portugal, due to the compromise imposed by hotter regional conditions. Morphology makes such compromises to multiple selection pressures easy to demonstrate (see Lowell, 1987), however, compromises to multiple stressors are likely to be present in any species which lives in a highly stressed environment (comprising either physical or biological stresses), (see Stafford et al., 2014).

Adapting to temperature at the expense of adaptation to other stressors, as appears to be occurring at lower latitudes in this study, may result in reduced ability to compete for resources or respond to other stresses (see discussion in Pandolfi et al., 2011). Although shore aspect and other features such as microhabitat availability can affect the actual temperature pressures faced by organisms (Denny et al., 2011), when near to species temperature limits, these shore features may not be enough to negate the negative effects of temperature on the performance of the organisms (Lima et al., 2016). As such, at these range limits, or on patches of shore approaching these limits, where there are few shaded areas or microhabitats, temperature stressed organisms, non-optimally adapted to other physical or biological stresses, may suffer from 'invasion' from range shifting species or other disruptions to community dynamics (Peterson et al., 2014; Pecl et al., 2017).

The current study does not consider the full range of *Patella depressa*, which can be found between SW England in the north through to Senegal in the south (Fischer-Piètte 1935), and as such the discussion about range limits is not directly applicable to the results from Portugal. However, this study does demonstrate that temperature changes the observed shell morphology of limpets, and this change in morphology appears to be related to characteristics of the shore on which the limpet lives. As such, the species becomes less optimally morphologically adapted to the shore as it moves south, even if this is well within its observed range. Essentially, this is a similar process, albeit at a very different geographical scale, as occurs in vertical distribution patterns of species (zonation) within a shore, with many species being higher on the shore than their morphological or physiological optimum would locate them (Hawkins and Hartnoll, 1985).

However, while not at the edge of their range, the current study also has relevance to early warning of biological responses of climate change. A typical response of climate envelope models to climate change is that the physical niche parameters of many species will shift polewards (Walther et al., 2002). However, in the majority of locations it is likely that the climate will get warmer; this is essentially equivalent to studying a similar habitat or community at a lower latitude. As such, monitoring limpet morphology for changes at specific and fixed locations over time therefore may provide a useful early warning for potential ecological change as a response to climate change.

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Table 1. Relative physical and biological stresses and stress reducing features of sites used in the study. With the exception of the first four columns, data are percentage contribution of stress type to the shore. Figures in bold illustrate high levels of certain factors associated with the k-means classification. For example, figures in bold in the Wave Index column illustrate the sites with the highest values of wave action tend to classify as type 2.

Site	Location	k-means Classification	Log$_4$ Sun/Wave ratio	Wave Index	Sun index	*Patella depressa*	Other grazers (including other limpets)	Mussels and Barnacles	Geological shelter (rockpools, cracks and crevices)	Average Nearest Neighbour	Complexity	Tidal height
1	N Spain	2	-0.5	9	3	16	6	16	**15**	6	13	16
2	N Spain	1	**0.29**	9	10	5	23	17	9	6	10	11
3	N Spain	3	0	**19**	15	14	1	4	5	15	13	15
4	N Spain	1	**0.29**	9	10	5	18	14	9	14	10	10
5	W Spain	1	**0.5**	6	9	9	21	18	2	15	11	9
6	W Spain	1	**0.79**	6	14	11	15	14	2	15	13	9
7	W Spain	3	-0.29	**20**	10	13	1	8	9	12	13	14
8	W Spain	1	**0.21**	12	12	4	23	16	7	12	9	5
9	Portugal	3	-0.21	**23**	13	12	1	2	7	18	12	12
10	Portugal	2	0	14	11	22	2	3	**24**	4	9	12
11	Portugal	1	**0.79**	5	11	9	14	14	15	11	11	9
12	Portugal	2	**1**	6	18	13	2	3	**21**	13	13	11

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Figure 1. Locations of the 12 sampling sites in Spain and Portugal. Note, differing scales in the inserts for each region

Figure 2. Mean (+/- 95% C.I.) pointedness values of limpets at different shore classifications (based on k-means clustering) and broad scale geographical locations. Note, there are no shore type 2 classifications for West Spain

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Figure 3. Mean (+/- 95% C.I.) pointedness values of limpets against the $\log_4$ transformed sun:wave ratio for 12 different sites. Lines of best fit are included for sites within each broad geographical region
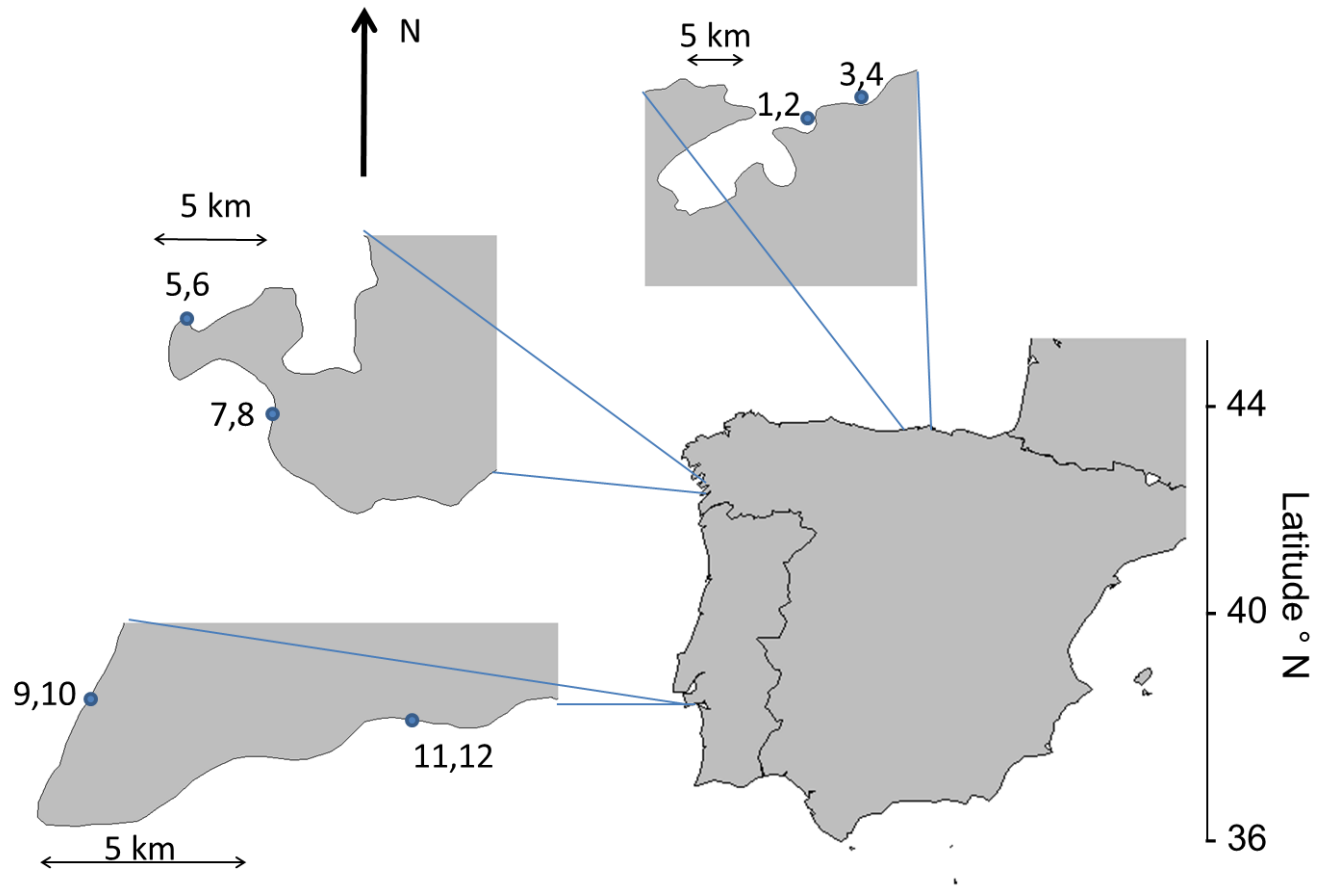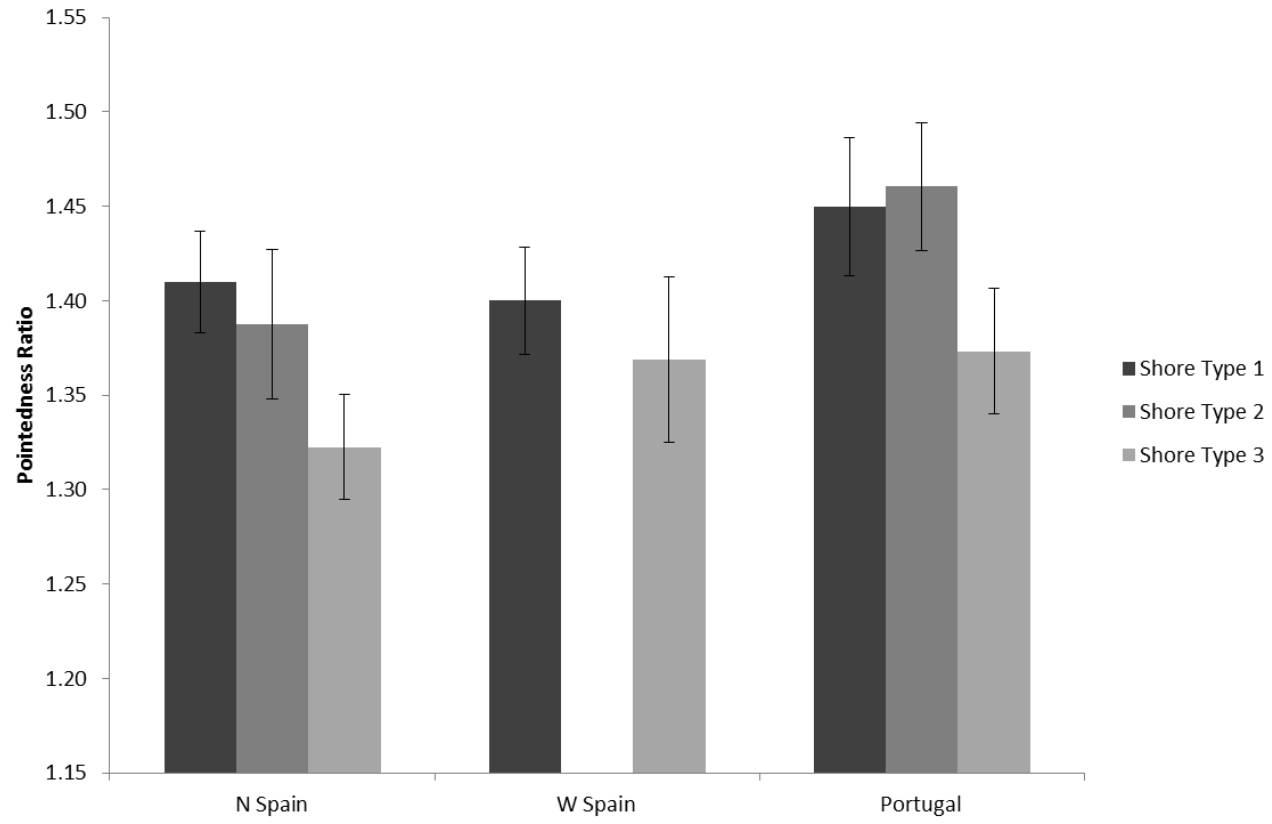
375   Fig 1

Fig 2

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Fig 3