Broad-scale patterns of sex ratios in *Patella* spp.: a comparison of range edge and central range populations in the British Isles and Portugal

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Sex change, or sequential hermaphroditism, occurs in the plant and animal kingdoms and often determines a predominance of the first sex. Our aim was to explore changes in sex ratios within the range of the species studied: Patella vulgata *and* Patella depressa. *The broad-scale survey of sex with size of limpets covered a range of latitudes from Zambujeira do Mar (southern Portugal) to the English Channel. Indirect evidence was found for the occurrence of protandry in* P. vulgata *populations from the south of England, with females predominating in larger size-classes; cumulative frequency distributions of males and females were different; sex ratios were biased towards males and smallest sizes of males were smaller than the smallest sizes of females. In contrast in Portugal females were found in most size-classes of* P. vulgata. *In* P. depressa *populations from the south coast of England and Portugal females were interspersed across most size-classes; size distributions of males and females and size at first maturity of males and females did not differ.* P. depressa *did, however, show some indications of the possibility of slight protandry occurring in Portugal. The test of sex ratio variation with latitude indicated that* P. vulgata *sex ratios might be involved in determining the species range limit, particularly at the equatorward limit since the likelihood of being male decreased from the south coast of England to southern Portugal. Thus at the southern range limit, sperm could be in short supply due to scarcity of males contributing to an Allee effect.*

Keywords: limpets, protandry, range distribution, sex change, sex ratio

INTRODUCTION

The abundance and distribution of a species throughout its geographic range are influenced by several ecological processes (Brown, 1995; Gaston, 2003, 2009; Sagarin *et al.*, 2006) including rates of reproductive output and recruitment success affecting demography (Hyder *et al.*, 2001), habitat quality and availability (Keith *et al.*, 2011), dispersal (Gaines *et al.*, 2009), competition with other species (Poloczanska *et al.*, 2008), plus predation including human harvesting (Fenberg & Rivadeneira, 2011). Differences in climate and hence environmental regime experienced will also influence survival, growth and reproduction of individuals and hence population processes (see Mieszkowska *et al.*, 2006, 2007; Moore *et al.*, 2011). At geographic range limits, population viability can be determined by physical barriers to dispersal (Gaines *et al.*, 2007; Lasky *et al.*, 2011), habitat quality (Herbert *et al.*, 2003; Herbert & Hawkins, 2006), biological

interactions such as competition (Poloczanska *et al.*, 2008) and predation (Sanford, 2002).

Little is known about how the above mechanisms differentially affect species in different parts of their range (e.g. equatorward and poleward limits *vs.* the centre) and how they relate to abundance and population structure across the range (Fenberg & Rivadeneira, 2011). The abundance and range limits of species may be reflected in population structure, life history and genetic traits (see Gilman, 2005, 2006; Gaston, 2009; Herbert *et al.*, 2009; Dawson *et al.*, 2010; Rivadeneira *et al.*, 2010). Low abundances near range limits reflect recruitment limitation, potentially caused by Allee effects or habitat availability (Zacherl *et al.*, 2003; Gilman, 2006; Sexton *et al.*, 2009) or poor larval supply due to hydrographic factors (Gaines *et al.*, 2007). In exploited species such as limpets, these patterns might be masked by the impact of size-selective human predation (Fenberg & Roy, 2012).

Theoretical models predict excesses of the first sex for species with sequential sex change (Charnov, 1982; Frank & Swingland, 1988; Charnov & Bull, 1989a, b). From sex-allocation theory, protandry (sequential sex change from male to female) is evolutionarily stable if female fertility increases with age faster than male fertility (Charnov & Bull, 1989b). Protandry is widespread in patellid limpets of the

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genera *Patella, Cymbula* (Orton, 1919, 1928, 1946; Bacci, 1955, 1975; Dodd, 1956; Orton *et al.*, 1956; Montalenti, 1958; Branch, 1974; Frenkiel, 1975; Espinosa *et al.*, 2009; Rivera-Ingraham *et al.*, 2011) and *Lottia* (Lindberg & Wright, 1985; Wright, 1989; Fenberg & Roy, 2012). In most studies protandry has been inferred from analysis of size-frequency data of single populations showing that females predominate in larger size-classes, although repeated biopsies of the same individual have tracked sex change in *Lottia gigantea* (Gray in G. B. Sowerby I, 1834) (Wright & Lindberg, 1979, 1982), *Patella vulgata* (Linnaeus, 1758) (Le Quesne & Hawkins, 2006) and *Patella ferruginea* (Gmelin, 1791) (Espinosa *et al.*, 2009; Guallart *et al.*, 2013). Little work has been done on variation between populations, especially on a geographic scale including their range edges (but see work on calyptraeids by Collin (2006) and on *P. ferruginea* by Rivera-Ingraham *et al.* (2011), showing considerable variation in sex ratio).

Previous studies have confirmed that size and time of sex change is sensitive to a range of factors (Munday *et al.*, 2006). These include: immediate social environment in limpets (Warner *et al.*, 1996; Collin *et al.*, 2005), snails (Chen *et al.*, 2004), shrimp (Baeza & Bauer, 2004) and many species of fish (Shapiro, 1984; Warner, 1984; Ross, 1990; Kuwamura & Nakashima, 1998); size of an individual relative to others in the social group (Warner & Swearer, 1991; Warner *et al.*, 1996; Buston, 2003); sex ratio of the social group (Shapiro, 1984; Munday, 2002; Collin *et al.*, 2005) and local density (Wright, 1989; Lutenesky, 1994).

Our overall aim was to investigate proportions of different sexes (as an indicator of protandry) in different size classes across multiple populations in the British Isles and Portugal. Specifically we compared the protandric *P. vulgata* at its southern range limit in Portugal, with populations at the centre of their distribution in the British Isles. Conversely, *Patella depressa* (Pennant, 1777) which is considered non-protandric in the UK (Dodd, 1956; Orton & Southward, 1961) was compared at its northern limit in the British Isles with populations at the centre of the range in Portugal. The Portuguese and English coasts respectively provide a sharp gradient of abundance of *P. vulgata* approaching its equatorward limits and *P. depressa* approaching one of its poleward range edges in the English Channel (Fischer-Piette, 1935; Crisp & Southward, 1958; Fischer-Piette & Gaillard, 1959; Southward *et al.*, 1995; Boaventura *et al.*, 2002b). Our survey was intended to explore the following alternative hypotheses:

1. The increasingly sub-optimal environment at range edges leads to slower growth and hence delayed promotion from male to female. Sub-optimal conditions could be due to the environment or increased competition from congeneric species (see Boaventura *et al.*, 2002a) at the centre of their range (i.e. *P. depressa* in Portugal, *P. vulgata* in the British Isles). The consequences of this hypothesis being correct would be that Allee effects due to the absence of females could be important in setting range limits. This assumes that interspecific competition is stronger than intraspecific competition.

2. Relaxation of resource limitation resulting from reduced intraspecific competition at lower density (see Boaventura *et al.*, 2003) will allow earlier promotion from males to females resulting in more females at lower densities (i.e.

sex change is density dependent). Thus we explored relationships between limpet density (single and both species) and sex ratio.

3. At range edges recruitment is intermittent and in protandric species this leads to older, large animals which are female. This could lead to Allee effects due to fewer males and hence potential sperm limitation.

In addition we wanted to confirm that *P. depressa* does not exhibit protandry further south in its range. Without manipulative experiments it is impossible to unambiguously test these alternative hypotheses. However, the patterns observed should provide further inference enabling hypotheses (1) to (3) to be ruled likely or unlikely.

MATERIALS AND METHODS

Geographic distributions and basic biology of study species

The two limpet species, *Patella vulgata* and *Patella depressa* are found in both the British Isles and Portugal. *Patella vulgata* occurs from the Arctic Circle near Tromsø in Norway to its southern biogeographic limit in southern Portugal (reviewed in Southward *et al.*, 1995). *Patella vulgata* is also present throughout Britain and Ireland (Southward *et al.*, 1995) and is considered a protandric species (Orton *et al.*, 1956), confirmed by repeated sampling of the same individuals (Le Quesne & Hawkins, 2006).

Patella depressa is restricted to the south and west coasts of Britain, with its northern biogeographic limit reached in North Wales, on Anglesey (Bowman & Lewis, 1986; Southward *et al.*, 1995; Kendall *et al.*, 2004). A single specimen was recently found on the Isle of Man (Hawkins unpublished observation). Eastern limits in the English Channel were the Isle of Wight, probably set by habitat availability, exposure and hydrography influencing larval supply (Crisp & Southward, 1958; Lewis, 1964, 1986; Keith *et al.*, 2011). Recently a small range extension has occurred on the sea defences at Hayling Island and Elmer near Bognor Regis (Hawkins, *pers. comm.*; Mieszkowska *et al.*, 2006; Keith *et al.*, 2011). *Patella depressa* is absent from Ireland (Southward & Crisp, 1954). In France, *P. depressa* does not occur beyond Cap de la Hague. They extend south to Senegal, West Africa (Southward *et al.*, 1995). *Patella depressa* has been considered a gonochoristic species in the UK (Dodd, 1956; Orton & Southward, 1961) but some size frequency data suggest the possibility of protandry on the Basque coast (Peña-Othaiz, 1996).

Study sites

The study was made on the south coast of England and in Portugal from September to November 2012 (Figure 1) when the *Patella* spp. were reproductively active enabling sex determination. Sites were chosen up to the range edge of the species in both England and Portugal.

Data collection

At each site in England and Portugal, 250 limpets were collected using 0.5 × 0.5 m quadrats from the mean tide level
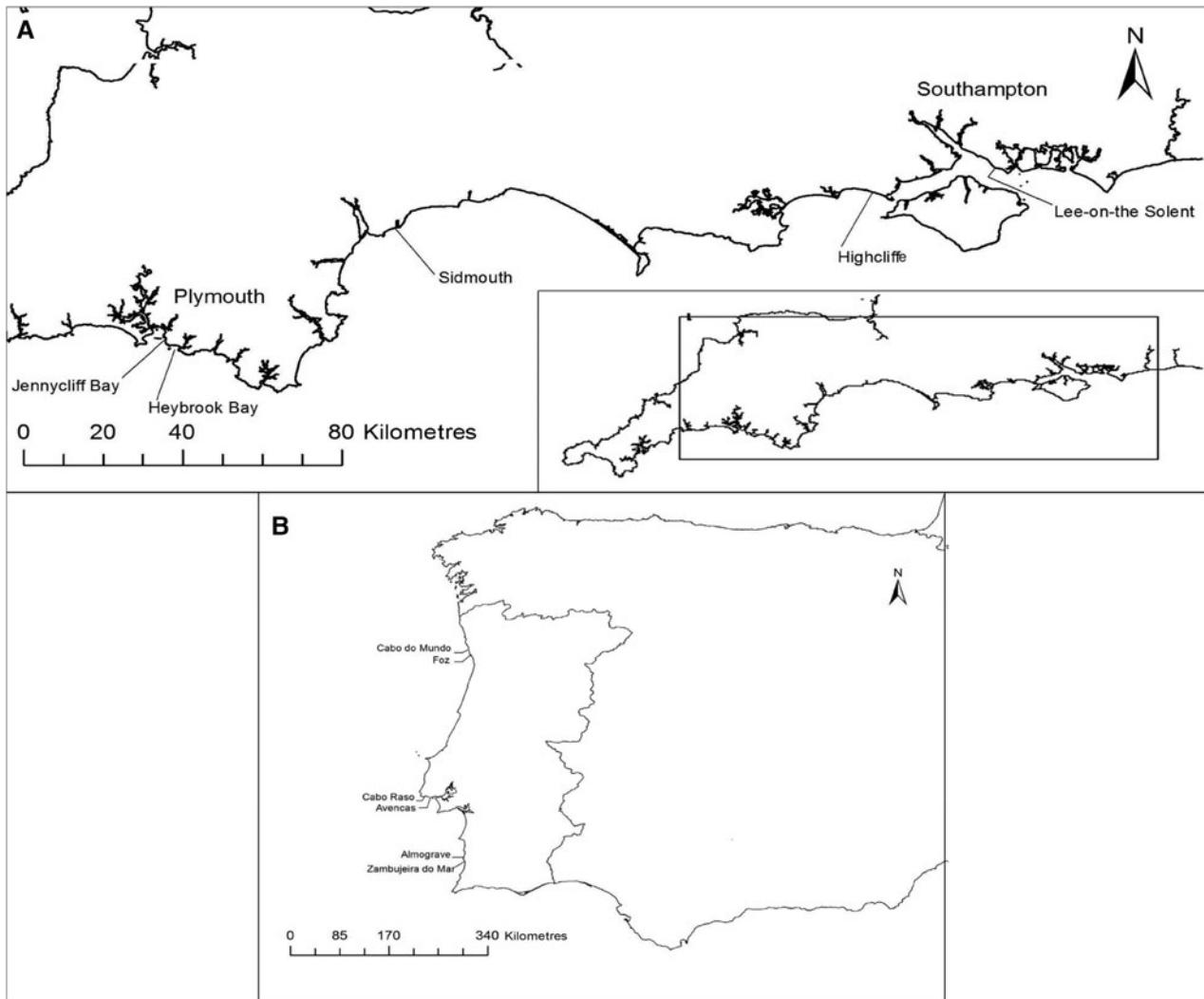
Fig. 1. (A) Map showing the south coastline of England and relative position of the five study site locations. Inset map shows the position of sites in the south of England. (B) Map showing the coastline of Portugal and relative position of the six study site locations.

(the spatial coordinates for each site are presented in Table 1). In England, Jennycliff and Heybrook Bay, being respectively a moderately sheltered and a moderately exposed site, were compared to assess local differences. Data for Highcliffe were duplicated on different groynes (Highcliffe 1 and Highcliffe 2) in order to check spatial variability in the collected data.

SIZE-FREQUENCY DISTRIBUTIONS AND SEX RATIOS
In the laboratory the collected limpets were separated by species, their maximum length measured and dissected to identify sex and stage of reproductive activity. Cumulative size-frequency data were plotted by species and visually compared. Kolmogorov–Smirnov tests (Sokal & Rohlf, 1998) were performed on size distributions of males and females for each species from the sites in England and Portugal.

The overall proportion of females at each sampled site was calculated as the (number of females)/(number of males + number of females). It was expected that the protandric *Patella vulgata* would show a male biased sex ratio in contrast to gonochoristic *Patella depressa* that should have equal proportions of males and females.

STATISTICAL ANALYSIS: MALE/FEMALE SIZES
A two-factor split-plot design was used to test the hypothesis that in potentially protandric species males were smaller than

Table 1. Location names of sampled sites and geographic coordinates.

Location	Coordinates (latitude/longitude)
England (from West to East)	
Jennycliff Bay	50°21′N 04°07′W
Heybrook Bay	50°19′N 04°07′W
Sidmouth	50°40′N 03°17′W
Highcliffe	50°48′N 01°13′W
Lee-on-the Solent	50°48′N 01°41′W
Portugal (from South to North)	
Zambujeira do Mar	37°31′N 08°47′W
Almograve	37°38′N 08°48′W
Avencas	38°41′N 09°21′W
Cabo Raso	38°42′N 09°29′W
Foz	41°09′N 08°40′W
Cabo do Mundo	41°13′N 08°43′W

females on average in body size, measured as length. The model was applied to species from England and Portugal separately. The design had two levels of the fixed Sex factor (Sx), at each level of a random Site factor (St, with six levels for England and Portugal for *P. vulgata*; with five levels for England and six for Portugal for *P. depressa*). Replicate random quadrats (Qt, 50 for England and Portugal for *P. vulgata*; 26 for England and 58 for Portugal for *P. depressa*) were nested in each level of St. This design was analysed with the General Linear Model (GLM) model: $Y = Sx|Qt'$ (St'), where a prime represents a random factor, vertical line means 'crossed with' and parentheses mean 'nested in'. For each species and by location the models were the following: *P. vulgata* from England and Portugal: $Y = Sx_2|Qt_{50}'(St_6')$; *P. depressa* from England: $Y = Sx_2|Qt_{26}'(St_5')$; *P. depressa* from Portugal: $Y = Sx_2|Qt_{58}' (St_6')$. During all analyses a visual inspection of the residuals was performed to check for the underlying assumptions of normality and homoscedasticity.

Additionally the smallest male and female individuals for each sampled population were calculated as the lowest 2.5% quantile for each size-frequency data and compared with a two-sample permutation test.

SEX RATIOS AND DENSITY OF SINGLE SPECIES AND ALL PATELLA SPP. COMBINED

The density of limpets by site was calculated as the number of individuals per quadrat and data were presented for each species (to explore possible intraspecific competition) and total species (to explore possible interspecific competition). Sex change was expected to be enhanced by low densities therefore the proportion of females was plotted against species density (intraspecific) and total species density (interspecific).

SEX RATIO VARIATION WITH LATITUDE

For each species a global binary logistic regression of sex *vs.* site (St, two factor level) and location (L, four level factor) was run to investigate the influence of latitude on the sex ratio. Sites ranged northwards from Portugal (Alentejo, Zambujeira do Mar and Almograve sites; Cascais, Avencas and Cabo Raso sites; Porto, Foz and Cabo do Mundo sites) to Plymouth, Jennycliff and Heybrook Bay sites on the south coast of England.

RESULTS

Size-frequency distributions and sex ratios

Size-frequency distributions for *Patella vulgata* (see Figure 2) along the English Channel accorded with the predictions of protandry, since larger individuals were predominantly female, although some large males were found. Size-frequency distributions of *P. vulgata* from Portugal did not show this pattern (see Figure 2). Cumulative size frequency data indicated that recruitment, assessed by the number of juveniles encountered, was also more pronounced in England than in Portugal, except for the artificial stone groyne at Lee-on-the-Solent which lacked suitable limpet nursery areas such as rockpools.
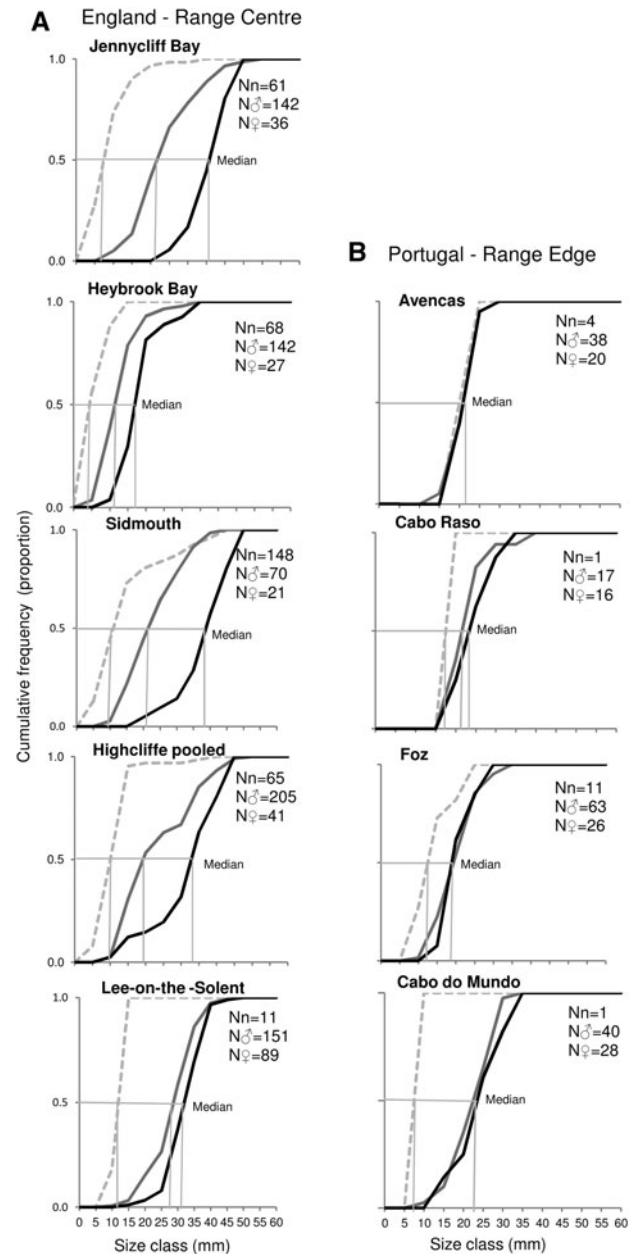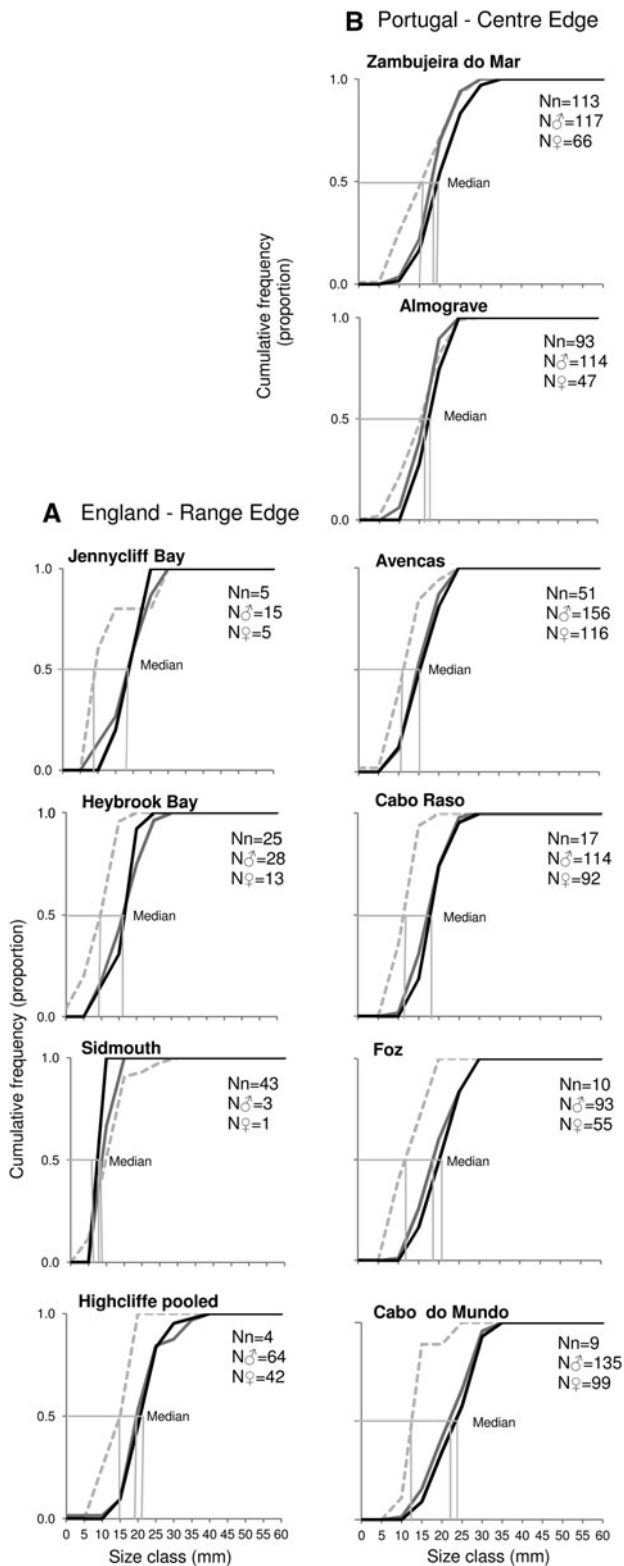

Fig. 2. Cumulative size frequencies for *P. vulgata* neuters (grey broken line), males (grey line) and females (black line) from range centre sample sites in (A) the south of England (Jennycliff Bay, Heybrook Bay, Sidmouth, Highcliffe (note: data from Highcliffe were pooled) and Lee-on-the-Solent) and from range edge sample sites in (B) Portugal (Avencas, Cabo Raso, Foz and Cabo do Mundo). Median size and abundance (N) of neuters (n), males (♂) and females (♀) are also indicated.

Patella depressa both in England and Portugal showed sexes interspersed in most of the size-classes suggesting a gonochoristic life history (see Figure 3).

The Kolmogorov–Smirnov tests indicated that for *P. vulgata* from England male and female size distributions differed from each other ($P < 0.05$) (Figure 2). For this species the cumulative frequency curves also showed that the median size of immature neuters was smaller than the median size of males and these median sizes were smaller than the median sizes of females. For *P. vulgata* from Portugal and *P. depressa* from both England and Portugal, male and female distributions did not differ from each other

Fig. 3. Cumulative size frequencies for *P. depressa* neuters (grey broken line), males (grey line) and females (black line) from range edge sample sites in (A) the south of England (Jennycliff Bay, Heybrook Bay, Sidmouth and Highcliffe (note: data from Highcliffe were pooled)) and from range centre sample sites in (B) Portugal (Zambujeira do Mar, Almograve, Avencas, Cabo Raso, Foz and Cabo do Mundo). Median size and abundance (N) of neuters (n), males (♂) and females (♀) are also indicated.

($P > 0.05$) (Figures 2 & 3). The cumulative frequency curves also showed that the median sizes of males were equal or very similar to the median sizes of females.

The proportion of females varied considerably between sites and between species (Figure 4). However, *P. vulgata* from England showed lower proportions of females than in Portugal which was confirmed by a two-sample permutation test ($P = 0.009$, N = 6 sites per sample). For *P. depressa* from England and Portugal no differences were detected on a two-sample permutation test for the proportion of females ($P = 0.238$, N = 5 sites per sample).

Male/female sizes

In southern England *P. vulgata* females were larger than males as also shown by the cumulative frequency curves and Kolmogorov–Smirnov tests (Figure 5). The GLM analysis of *P. vulgata* mean body size revealed a significant sex × site interaction (Table 2: $F_{5, 38} = 4.60$, $P = 0.002$) and main effects of sex and site (Table 2: $F_{1, 5} = 27.32$, $P = 0.003$, and $F_{5, 44} = 12.60$, $P < 0.001$ respectively). The overall smaller size of males than females therefore had a site-dependent magnitude of difference. For *P. vulgata* from Portugal (Figure 5, Table 2) the GLM detected no differences by sex or by site.

For *P. depressa* from England differences were only detected by site (Figure 5, Table 3: $F_{4, 21} = 6.69$, $P = 0.001$) while for *P. depressa* from Portugal differences were detected by sex (Figure 5, Table 3: $F_{1, 5} = 49.84$, $P = 0.001$) and site (Figure 5, Table 3: $F_{5, 52} = 13.58$, $P < 0.001$). This difference in mean sizes by sex could be indicative of some protandry occurring in *P. depressa* populations from Portugal.

In the southern England, males of *P. vulgata* were smaller than the females (Figure 6). This was confirmed by a two-sample permutation test for the smallest sizes of males and females ($P = 0.042$, N = 6 sites per sample). For *P. vulgata* from Portugal (Figure 6) no differences were detected on a two-sample permutation test for the smallest sizes of males and females ($P = 0.091$, N = 6 sites per sample).

For *P. depressa* from England and Portugal (Figure 6) no differences were detected on a two-sample permutation test for the smallest sizes of males and females ($P = 0.778$ and $P = 0.082$, N = 5 and N = 6 sites per sample respectively).

Sex ratios and densities of single species and all *Patella* spp. combined

Densities of *P. vulgata* were higher in England than in Portugal while *P. depressa* had higher densities in Portugal than in England (Figure 7). In England, *P. vulgata* showed greater variability in densities. The moderately exposed site at Heybrook Bay had higher numbers of individuals of both *P. vulgata* and *P. depressa* than the moderately sheltered site at Jennycliff Bay.

In England, the total density of *Patella* spp. was higher on the more exposed sites (Figure 7). The low density at Lee-on-the-Solent probably reflected the lack of nursery grounds such as pools and crevices (Bowman & Lewis, 1977) on an artificial structure (see Moschella et al., 2005; Firth et al., 2013). In Portugal, the density of *Patella* spp. was higher on southern than northern sites (Figure 7).

The pooled data from England and Portugal indicated that the proportion of females decreased with increasing density of
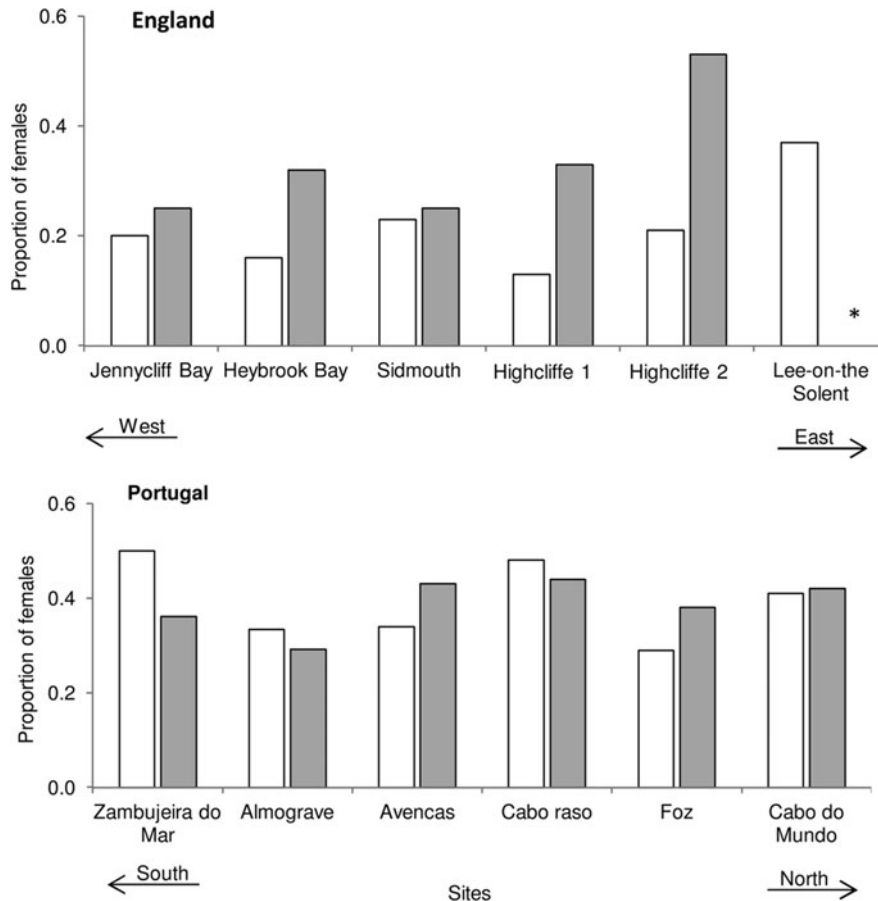
Fig. 4. Proportion of females of *P. vulgata* (clear bars) and *P. depressa* (grey bars), from the sample sites in the south of England (250 animals were collected from N = 5–12 quadrats per site) (* *P. depressa* absent) and Portugal (250 animals were collected from N = 4–17 quadrats per site).

P. vulgata, but not with increasing density of all *Patella* spp. (Figure 8, top left: $r^2 = 0.60$, $F_{1,10} = 15.14$, $P = 0.003$ and Figure 8, top right: $r^2 = 0.04$, $F_{1,10} = 0.45$, $P = 0.519$ respectively). The proportion of females of *P. depressa*, in contrast,

showed no relationship with density of *P. depressa* alone, nor all *Patella* spp. combined (Figure 8, bottom left: $r^2 = 0.001$, $F_{1,9} = 0.01$, $P = 0.913$ and Figure 8, bottom right: $r^2 = 0.15$, $F_{1,9} = 1.64$, $P = 0.233$ respectively).
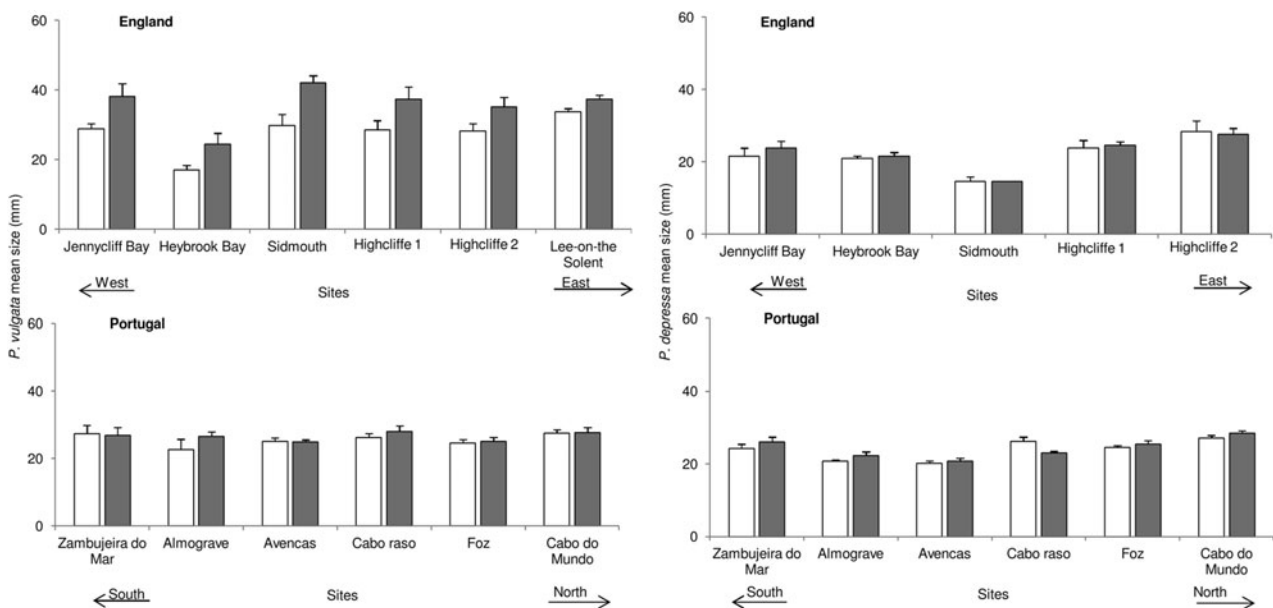

Fig. 5. Mean sizes (mm) (+SE) of *P. vulgata* and *P. depressa* males (clear bars) and females (grey bars) from the sample sites in the south of England (250 animals were collected from N = 5–12 quadrats per site) and Portugal (250 animals were collected from N = 4–17 quadrats per site).

Table 2. *Patella vulgata* GLM on mean size (mm) by sex (Sx) and site (Sf) in sample quadrats (Qf) in the south of England (a) and Portugal (b). Terms with $P < 0.05$ are in bold.

Source	d.f.	Seq SS	Seq MS	F	P
(a) England					
Between quadrats[a]					
St′	5	2175.19	435.04	12.60	**<0.001**
Qt′(St′)	44	1519.78	34.54	–	
Within quadrats[b]					
Sx	1	1635.14	1635.14	27.32	**0.003**
Sx × St′	5	299.24	59.85	4.60	**0.002**
Error	38	494.70	13.02		
(b) Portugal					
Between quadrats					
St′	5	147.03	29.41	2.16	0.076
Qt′(St′)	44	599.77	13.63	–	
Within quadrats					
Sx	1	10.40	10.40	1.93	0.223
Sx × St′	5	26.93	5.39	0.33	0.892
Error	37	599.19	16.19		

[a]Sources of variation measured from replicate quadrats.
[b]Sources of variation measured from replicate individuals within quadrats.

Table 3. *Patella depressa* GLM on mean size (mm) by sex (Sx) and site (St) in sample quadrats (Qt) in the south of England (a) and Portugal (b). Terms with $P < 0.05$ are in bold.

Source	d.f.	Seq SS	Seq MS	F	P
(a) England					
Between quadrats					
St′	4	583.39	145.85	6.69	**0.001**
Qt′(St′)	21	457.14	21.77		
Within quadrats					
Sx	1	14.74	14.74	4.27	0.108
Sx × St′	4	13.80	3.45	0.22	0.926
Error	16	255.74	15.98		
(b) Portugal					
Between quadrats					
St′	5	748.83	149.77	13.58	**<0.001**
Qt′(St′)	52	573.73	11.03	–	
Within quadrats					
Sx	1	35.89	35.89	49.84	**0.001**
Sx × St′	5	3.61	0.72	0.27	0.930
Error	51	137.96	2.71		

Sex ratio variation with latitude

For *P. vulgata*, the odds of being male decreased with decreasing latitude, reaching a maximum value of 3.94 in Plymouth relative to Alentejo in southern Portugal (Table 4: $Z = 2.08$, $P = 0.037$). For *P. depressa* in contrast, the odds ratios show no trend with latitude ($|Z| < 1.62$, $P > 0.1$ for all comparisons; table not presented).

DISCUSSION

Protandry

Our survey indicated that protandry appears to be occurring in *Patella vulgata* from the southern English coast, but not in *Patella depressa*. There were, however, indications of the possibility of some protandry occurring also for *P. depressa* from Portugal: female mean sizes were larger than male

mean sizes (Peña-Othaiz, 1996). Interestingly sex ratios of *P. vulgata* in Portugal were less male biased than in England.

There is strong indirect evidence of the occurrence of protandry in *P. vulgata* populations from the south of England: females predominated in larger size-classes; cumulative frequency distributions of males and females were different; sex ratios were biased towards the first sex and smallest sizes of males were smaller than the smallest sizes of females. For *P. vulgata* populations from Portugal no such patterns were found: females were interspersed across most size-classes; cumulative frequency distributions of males and females did not differ; the proportion of females was higher than in England and smallest sizes of males and females did not differ.

The above observations raise the possibility that *P. vulgata* from Portugal were not protandric. The observed patterns could, however, be most simply explained as the result of low recruitment occurring in Portugal, generating populations with larger older females with few smaller males. As a consequence of poor recruitment these populations had low densities. An alternative explanation would be that due to low intraspecific competition, there is a higher incidence of males changing sex at smaller sizes. This would result in a higher proportion of females at low limpet densities (Figure 8). Previous studies on *P. vulgata* from England and Scotland indicated that sex ratios varied between populations (Das & Seshappa, 1948; Ballantine, 1961; Blackmore, 1969; Lewis & Bowman, 1975; Baxter, 1983). The sex ratios found ranged from male biased (Ballantine, 1961; Baxter, 1983) to numbers of males and females approximately equal (Das & Seshappa, 1948; Lewis & Bowman, 1975). In all populations the majority of small, mature individuals were male and the number of females increased with size. In Portugal, a predominance of females was found in *P. vulgata* samples from the northern and central coast (Ribeiro *et al.*, 2009) while previous studies had mentioned male-biased sex ratios (Guerra & Gaudêncio, 1986; Castro, 2004). In any case, the density especially of new recruits of *P. vulgata* seems to have more influence in determining female numbers suggesting that sex change could be density dependent (Figure 8), either due to direct food limitation (Hoagland, 1978) or cues from conspecifics (Collin *et al.*, 2005; Collin, 2006). Indeed, Rivera-Ingraham *et al.* (2011) found that size at sex change was correlated with density of larger individuals: scarcity of larger individuals promoted sex change at smaller sizes.

Low densities lead to an earlier promotion from males to females in *Lottia gigantea* with individuals being more likely to change sex in artificially lowered densities (Wright, 1989). Wright's (1989) field survey found that at low densities females occurred at younger ages. In a recent study, *L. gigantea* individuals from harvested populations changed sex at smaller sizes and grew at slower rates compared with individuals from protected populations (Fenberg & Roy, 2012). A similar mechanism was described for *Patella ferruginea*: individuals in populations with low density of larger individuals switched to female at smaller sizes (Rivera-Ingraham *et al.*, 2011; Guallart *et al.*, 2013). Such sex-change plasticity seems also to be occurring in *P. vulgata* when subjected to exploitation (Borges, 2013; Borges *et al.*, in submission).

Patella depressa both from England and Portugal exhibited patterns expected of a gonochoristic species: females were interspersed across most size-classes; cumulative frequency distributions of males and females and smallest sizes of
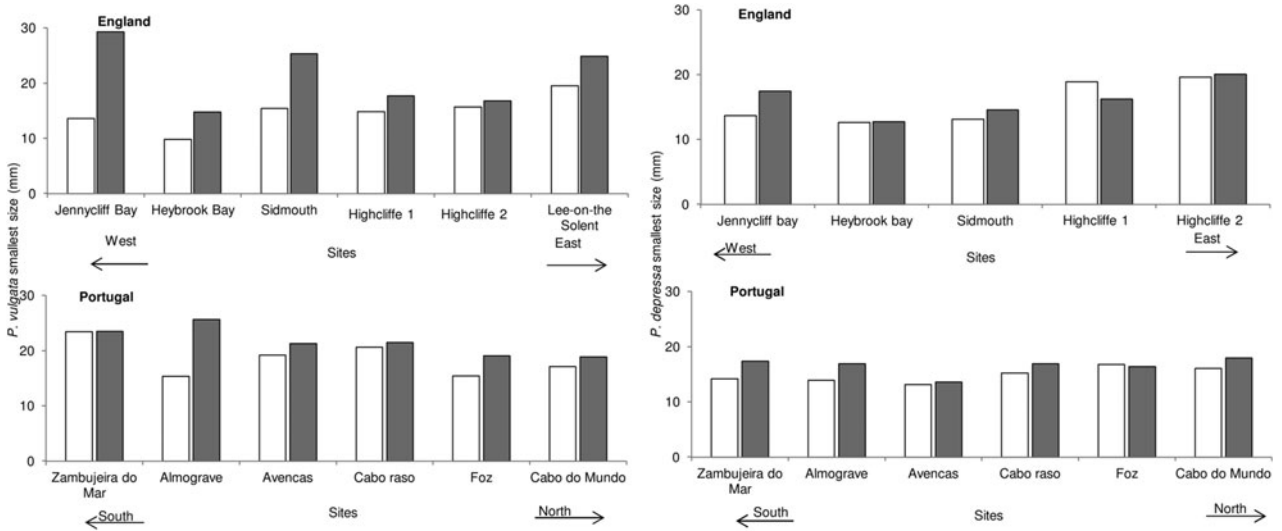
Fig. 6. Smallest sizes (mm) of *P. vulgata* and *P. depressa* males (clear bars) and females (grey bars) from the sample sites in the south of England (250 animals were collected from N = 5–12 quadrats per site) and Portugal (250 animals were collected from *n* = 4–17 quadrats per site).

males and females were similar. These results were partially corroborated by the GLM on body size: for *P. depressa* from England no differences in mean size between sexes was detected; while in Portugal size differed slightly by sex, which could be indicative of some protandry occurring in populations from Portugal. The plots of mean size and smallest size also suggest that for *P. depressa* from Portugal males were generally slightly smaller than females (Figures 5 & 6).
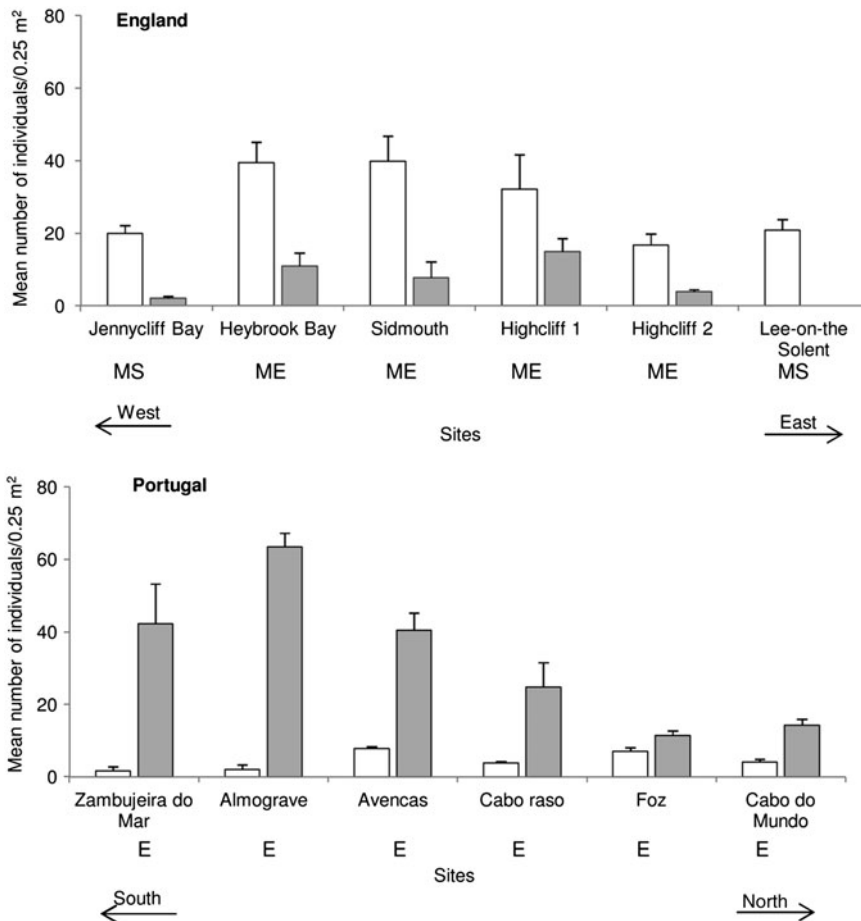
Fig. 7. Density (mean number of individuals/0.25 m²) (+SE) of *P. vulgata* (clear bars), *P. depressa* (grey bars) from the sample sites in the south of England (N = 5–12 quadrats per site) and Portugal (N = 4–17 quadrats per site). MS, ME and E refer to moderately sheltered, moderately exposed and exposed sites, respectively.
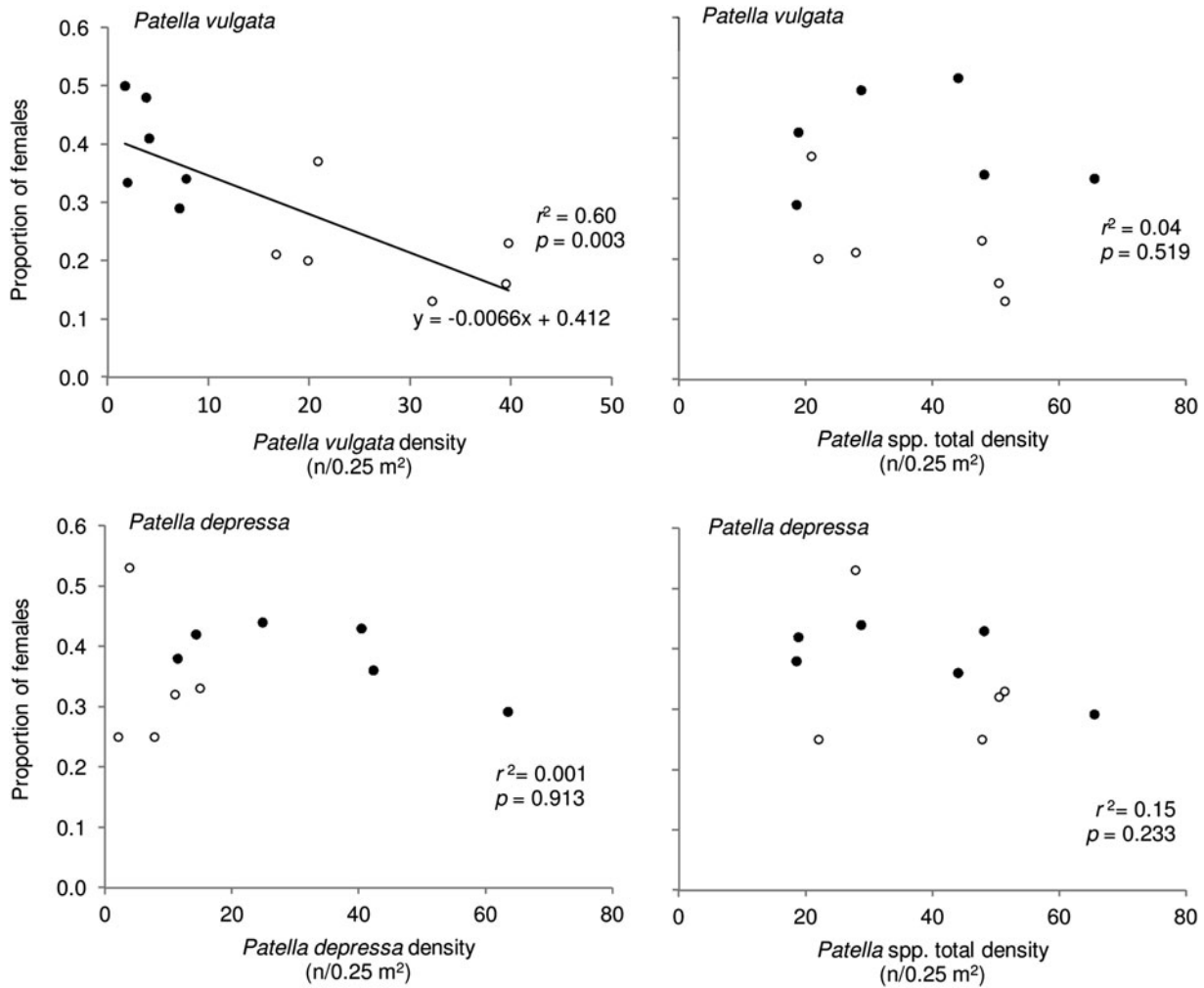
Fig. 8. *Patella vulgata* proportion of females by *P. vulgata* density and by *Patella* spp. density (number of individuals/0.25 m²) (top graphs) and *P. depressa* proportion of females by *P. depressa* density and by *Patella* spp. density (bottom graphs) for the sample sites in the south of England (○) and Portugal (●).

This could be due to differential growth or mortality between males and females rather than protandry or low recruitment. A predominance of males in *P. depressa* from Portugal was mentioned in previous studies (Guerra & Gaudêncio, 1986; Castro, 2004; Ribeiro *et al.*, 2009), although some data exist on equal numbers of males and females (Brazão *et al.*, 2003). In fact, Boaventura *et al.* (2003) suggested that the

higher percentages of males found in increased *P. depressa* density treatments could possibly be explained by a suppression of protandry due to competition. Thus the possibility of some protandry occurring in this species remains (see also Peña-Othaiz, 1996).

The variability encountered in sex ratios in other studies emphasizes that protandry should not be inferred solely from those data and it should be taken into consideration that there are partially sex-changing animals, where a proportion of the 'second' sex mature directly from the juvenile (early maturers), having never passed through the 'first sex' (Allsop, 2003). Studies should combine different analyses such as male/female distributions comparisons, microscopy, manipulative experiments and direct observation of individuals (see Wright & Lindberg, 1979, 1982; Le Quesne & Hawkins, 2006).

Geographic range edges

The distributions and relative abundances of *P. vulgata* and *P. depressa* were in accordance with the known range limits for these species. *Patella vulgata* was more abundant in the south of England and *P. depressa* was more abundant in Portugal.

Table 4. *Patella vulgata* global binary logistic regression of sex *vs.* site (two factor level with Zambujeira do Mar site from Alentejo as reference) and location (four factor level with Alentejo (Zambujeira do Mar and Almograve sites) as reference). Terms with $P < 0.05$ are in bold.

	Z	P	Odds ratio
Location			
Cascais	0.93	0.352	1.90
Porto	1.31	0.189	2.42
Plymouth	2.08	**0.037**	3.94
Site			
Almograve	0.65	0.518	2.00
L × St			
Cascais × Almograve	−1.10	0.272	0.28
Porto × Almograve	−1.09	0.277	0.29
Plymouth × Almograve	−0.37	0.715	0.67

In Portugal, *P. vulgata* densities increased from south to north while *P. depressa* numbers decreased. This accorded with the known distribution of both species: *P. vulgata* is a boreal cold temperature species; *P. depressa* is a southern warm water species (e.g. Southward *et al.*, 1995 for review).

The test of sex ratio variation with latitude suggested that distorted sex ratios of *P. vulgata* might be involved in setting the species southern range limit, since the likelihood of being male decreased with latitude from Plymouth (southern England) through Alentejo (southern Portugal). Hypotheses (2) and (3) could explain the higher proportions of females in populations from Portugal since resource relaxation due to lower intraspecific competition would allow higher rates of promotion of males to females; alternatively intermittent and low recruitment leave populations with few males and older larger females. In England, *P. vulgata* is at the centre of its range and sex change is probably occurring given the male-biased sex ratio found across those populations. A male-biased sex ratio has been observed for several protandric limpets (e.g. *Crepidula* spp.; Collin, 2006). If skewness in sex at lower latitudes compromises the species' reproductive success then Allee effects due to the reduction of the numbers of males could be occurring (e.g., Boukal & Berec, 2002; Kent *et al.*, 2003; Walker *et al.*, 2010; Lee *et al.*, 2011). This scarcity of males in free-spawners will result in reduced numbers of male gametes with low probability of contact with eggs. High sperm concentrations are known to be important in *P. vulgata* (Hodgson *et al.*, 2007). *Patella depressa* showed no tendency of change in sex ratio with latitude, therefore sex ratios possibly do not influence the species northern limit as it is known as not protandric in England.

Concluding comments

Of the three original hypotheses, hypothesis 1 (delayed promotion to females at range edges) can be rejected. There are indications that hypothesis 2 (promotion from male to female is density dependent) may apply to *P. vulgata*; but the observed correlation could be due to differences in the age structure due to limited recruitment at range edges. Parallel experimental work (Borges, 2013; Borges *et al.*, submitted) also supports this hypothesis. Hypothesis 3 (recruitment is intermittent at range edges) seems to be occurring in *P. vulgata* leading to limited numbers of males. This does not apply to gonochoristic species such as *P. depressa* at its range edge in the British Isles. Since we found some slight indirect evidence for the possibility of *P. depressa* being protandric in Portugal, it could be the case that this species has a variable life history depending on environmental and demographic constraints.

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