

Updated global distribution of the threatened marine limpet *Patella ferruginea* (Gastropoda: Patellidae): an example of biodiversity loss in the Mediterranean

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Abstract *Patella ferruginea* is the most threatened macro-invertebrate in the western Mediterranean, where it is at serious risk of extinction. There is little information on the status of the various populations and most data were published more than 25 years ago. This study provides updated information on the global status of this species, and implications for management and conservation, and tests the hypothesis that population structure can be influenced by collection by people and by the type of substrate. Fifty-five localities were surveyed in Corsica, Sardinia, Tuscany and Sicily, on the islands of Egadi and Pantelleria, and on the Zembra archipelago and Tunisian coasts. The species is extinct on mainland Italy and Sicily but isolated individuals can be found on Egadi and Pantelleria. Populations on Corsica and Sardinia have declined dramatically during the last 25 years. The population in the Zembra archipelago is well preserved, although more widely on Tunisian coasts the species is highly threatened. The information provided here, combined with information from the literature, indicates a strong decline and/or extinction of many populations throughout the western Mediterranean and the presence of healthy populations only in some locations along the north African coast. The species exhibits an increase in density and mean size in areas free of human pressure but the type of substrate (natural or artificial) has no strong influence.

Keywords Artificial substrate, conservation, distribution, limpets, Mediterranean, molluscs, *Patella ferruginea*

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Introduction

The Mediterranean Sea, home to many threatened and endemic species, has suffered a general decrease in biodiversity, with temporal trends indicating that over-exploitation and habitat loss have been the main drivers of this change and that these influences are expected to increase (Coll et al., 2010). Overexploitation on rocky shores involves people collecting animals and plants for food or bait (Keough et al., 1993); on the accessible intertidal areas there are many target species, such as limpets, that are extensively collected (Lindberg et al., 1998). An example is *Patella ferruginea*, the most threatened marine macro-invertebrate in the western Mediterranean (Ramos, 1998), which is included in several directives at European and country levels (MMAMRM, 2008). This species has been collected since the Pleistocene (Espinosa et al., 2009a). Exploitation can decrease the reproductive output of intertidal invertebrate populations because individual fecundity of many species increases with body size (Levitan, 1991; Tegner et al., 1996).

According to MMAMRM (2008) *P. ferruginea* occurs in North Africa (e.g. Ceuta, Melilla); the Chafarinas Islands (Spain); Al Hoceima Natural Park, Morocco; the Rachgoun and Habibas Islands (Algeria); the Cap Bon Peninsula and Zembra Island (Tunisia); and the southern Iberian Mediterranean coast, Corsica and Sardinia. However, there is little known about the status of these populations and most of the available information dates from >25 years ago. The only research addressing the global distribution of *P. ferruginea* was that of Laborel-Deguen & Laborel (1991a), although much of the data provided by them were from palaeological sources rather than direct census. It has been suggested that the increase in artificial structures in the Mediterranean could lead to a general loss of genetic biodiversity, populations and species (Fauvelot et al., 2009; Bulleri & Chapman, 2010). Nevertheless, several species, including *P. ferruginea*, have settled on artificial substrates, where dense populations can be found (García-Gómez et al., 2011). However, there is a lack of information about how the type of substrate influences the population dynamics of this species. The aim of this research was to provide updated information for the entire western Mediterranean, to evaluate the current status of this species. Additionally,

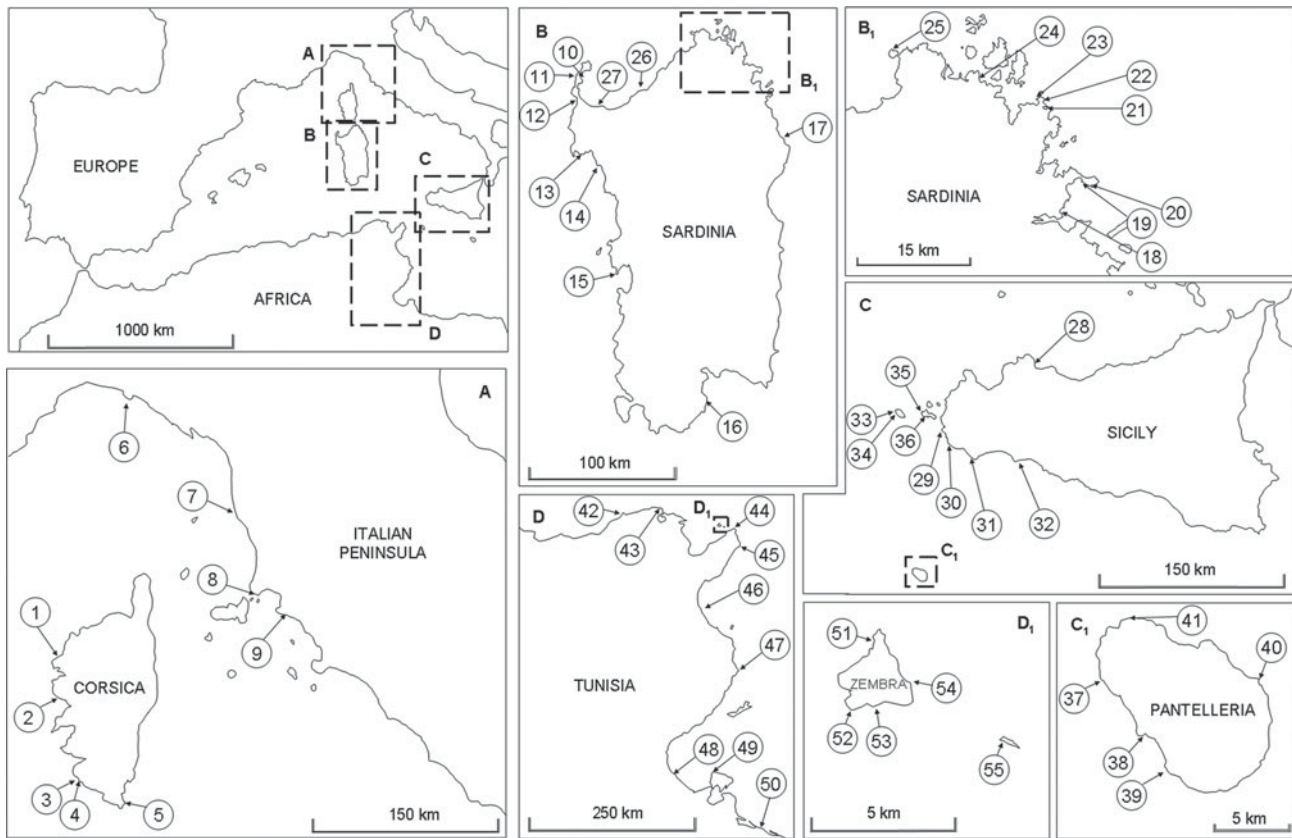


Fig. 1 Locations of the 55 study sites where we surveyed *Patella ferruginea*. For details of each site see Supplementary Table S1.

we tested the hypothesis that population structure and related reproductive success can be influenced by collection by people and by the type of substrate on which the populations are settled (artificial vs natural). The results will assist in the conservation management of this species.

Study area

We explored the shores of the mainland and of Zembra and Zembretta islands, Tunisia, Pantelleria and the Egadi islands off western Sicily, the western Sicilian mainland, Corsica, Sardinia and the Tuscany–Liguria coast of Italy (Fig. 1, Supplementary Table S1). We visited sites where the species had been recorded previously on the Egadi islands and Pantelleria (Giaccone & Sortino, 1974), along the Tuscany–Liguria coast (La Rochette: Curini-Galletti, 1979; Quercianella: Terreni, 1981; Piombino: Biagi & Poli, 1986; Portofino: Porcheddu & Milella, 1991) and on Corsica (Laborel-Deguen & Laborel, 1990, 1991b). We also collected all available information on the status of the species throughout the Mediterranean.

Methods

Sampling sites were selected on rocky habitats, where the species settles, mainly in marine protected areas or at

locations with healthy marine communities (because of the sensitivity of the species to pollution and collection; Espinosa et al., 2007). Transects parallel to the coast were surveyed at each locality, by snorkelling on steep or sloping shores and on foot on shallow sloping shores. All limpets settled on the shore were recorded. At localities with sparse populations 100 m transects were surveyed, whereas 10 m transects were used at sites with high densities (Supplementary Table S1; Laborel-Deguen & Laborel, 1991b; Guerra-García et al., 2004). All specimens of *P. ferruginea* found were measured with a calliper to the nearest mm along the longitudinal axis (Guerra-García et al., 2004). Small individuals are difficult to detect (Guallart et al., 2006) and therefore particular care was taken to record this component of the population. For graphical representations of size frequencies we have only taken into consideration sites with at least 10 specimens. Data from the sites surveyed in the Zembra archipelago were pooled because genetic analyses have shown that there is no limitation to gene flow in this area (Casu et al., 2012).

The assumptions for parametric statistics were not met by some of our data, with severe departures from normality and highly unequal variances. The ANOVA test is, however, robust for deviation from normality (Underwood, 1997) and if a Type I error is minimized (by using $P < 0.01$) it is possible to conduct an analysis even if the condition of

TABLE 1 Summary statistics for the size distribution of *Patella ferruginea* (only for sites where the species was detected). Site numbers (Supplementary Table S1; Fig. 1) are in parentheses.

Location (site)	n	% <3 cm	Max. size (cm)	Min. size (cm)	Mean size±SD (cm)	Skew±SE
Galeria (1)	40	47.5	6	0.6	3.14±1.15	0.046±0.374
Cargese (2)	10	20	6.2	1.9	4.16±1.32	-0.516±0.687
Tizzano (3–4)	4	75	5.6	1.6	2.97±1.78	1.735±1.014
Bonifacio (5)	1	100	2.4	2.4	2.4	
Asinara (10)	40	12.5	9.3	1.9	6.14±2.03	-0.724±0.374
Capo Falcone (11)	8	75	3.4	1.4	2.43±0.66	-0.012±0.752
Olbia (18)	11	36.33	3.8	1.4	2.97±0.71	-0.993±0.661
Cala Moresca (20)	1	0	4.9	4.9	4.9	
Porto Rafael (24)	19	47.36	6.6	0.4	3.05±1.63	0.295±0.524
Capo Testa (25)	36	8.33	6.6	1.9	4.36±1.05	-0.118±0.393
Punta Mugnona (33)	4	0	6.5	4	4.92±1.19	0.921±1.014
Grotta Presepe (34)	3	0	6.5	5.5	5.66±0.76	0.935±1.225
Punta Sottile (35)	3	33	4.6	2.1	3.23±1.26	0.801±1.225
Punta Rosso di Nica (39)	1	100	1.9	1.9	1.9	
El Haouaria (44)	16	100	2.6	1.2	2.08±0.40	-0.726±0.564
Kelibia (45)	16	68.75	4.6	0.9	2.57±0.89	0.239±0.564
Zembra (51–55)	850	27.17	8.8	0.3	4.29±2.26	-0.353±0.084*

*Skew is considered significant when its value is $>2 \times$ SE.

equal variances is not met. Differences between means were examined a posteriori using the Student–Newman–Keuls test. Multivariate MDS (non-metric multidimensional scaling) statistics were also used, based on the UPGMA method (unweighted pair-group method using arithmetic averages), along with the Bray–Curtis similarity index. MDS was used to test differences in population structure among sites (i.e. number of specimens in each 1-cm size class from 0 to 10 cm). Clusters of sites identified as statistically significant using the profile test SIMPROF ($P < 0.05$) were considered to have a similar population structure. Kruskal's stress coefficient was used to test the ordination (Kruskal & Wish, 1978). PERMANOVA (permutational multivariate analysis of variance) was used to test hypotheses regarding differences in population structure between types of substrate (natural vs artificial) and accessibility, assuming that at less accessible sites the limpets are more protected from collection (protected vs unprotected). Analyses were performed using a $\log(x+1)$ transformation for the MDS analysis and fourth-root transformation for the PERMANOVA test, to lessen the influence of the more abundant size classes. The abundance of each size class was standardized to density (individuals per m of transect) to avoid artefactual differences among populations resulting from the differing sampling effort at each site. Data for the MDS and PERMANOVA analyses were compiled from the present study, augmented by information from Granada and Alboran island (Spain; Barba et al., 2006), Crinavis (Algeciras Bay, Spain; Navarro-Barranco, 2010), San Felipe breakwater (Algeciras Bay, Spain; Espinosa et al., 2005), Ceuta (North Africa, Spain; Espinosa et al., 2009a), Habibas and Plane islands

(Algeria; Espinosa, 2009) and Melilla (North Africa, Spain; González-García et al., 2006). Univariate and multivariate analyses were carried out using SPSS v. 15.0 (SPSS, Chicago, USA) and PRIMER v. 6.0 (Clarke & Gorley, 2006).

Results

In total 1,063 individuals of *P. ferruginea*, distributed non-homogeneously (Supplementary Table S1; Table 1; Fig. 2), were recorded and measured. The species is extremely scarce on mainland Tunisia (Fig. 2e,f; in the Cap Bon area, although there are other small populations from the Gulf of Tunis to Monastir, see Discussion). A large and well-structured population was recorded in the Zembra archipelago (mean density 2.65 m^{-1}), with high levels of recruitment and large, reproductively mature specimens (Fig. 2b). On the Egadi islands we found four individuals (4.0, 4.0, 5.2, 6.5 cm in length) at Punta Mugnona, three at Grotta Presepe (5.0, 5.5, 6.5 cm; both sites on Marettimo Island), and three at Punta Sottile (2.1, 3.0, 4.6 cm; Favignana Island). Only one individual (1.9 cm) was recorded on Pantelleria, at Punta Rosso di Nica. On Corsica and Sardinia the populations were highly fragmented and densities were low, with only one individual (2.4 cm) found at Bonifacio and four at Tizzano (1.6, 2.2, 2.5, 5.6 cm), although some sites, such as Asinara (Sardinia), had more individuals (Fig. 2a). The species was absent from mainland Italy and Sicily. There were significant differences in mean shell length among populations, with the largest individuals at Asinara (Table 2).

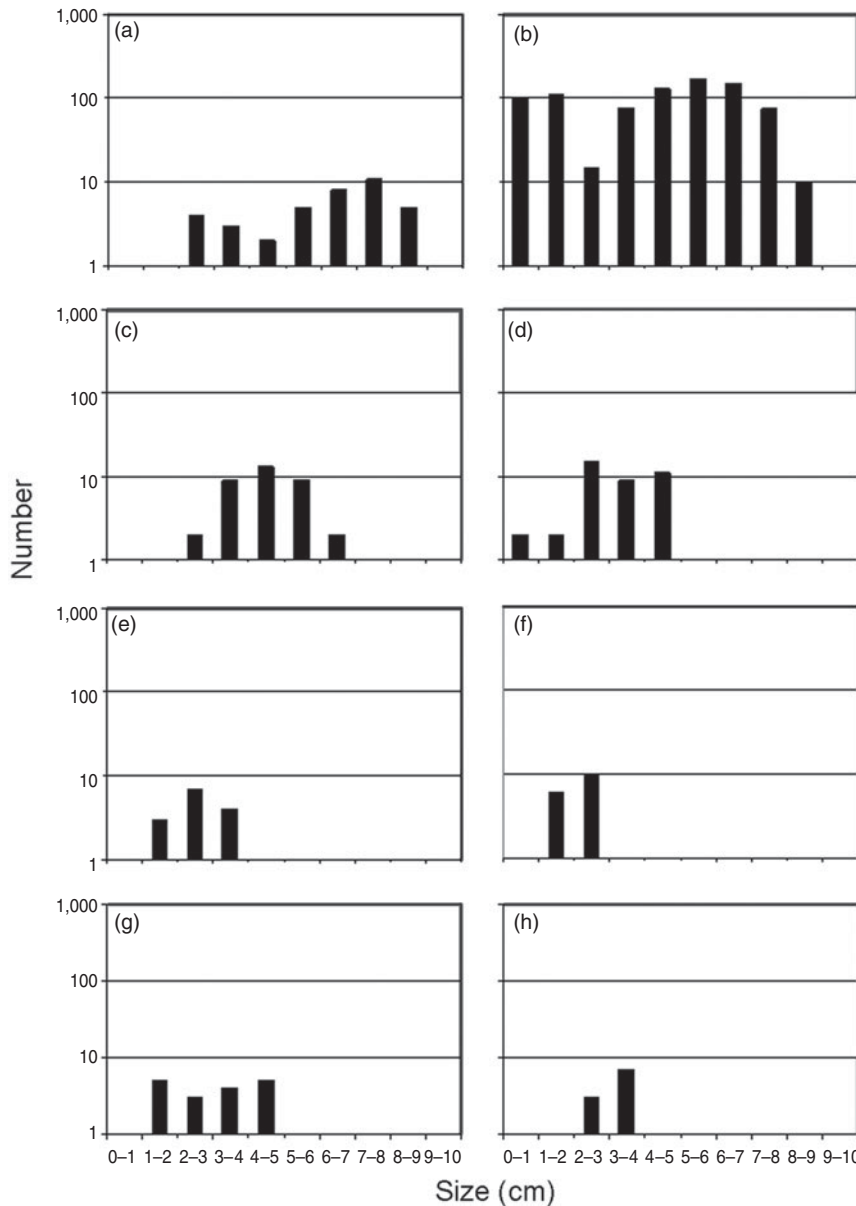


FIG. 2 Size frequency distribution of *P. ferruginea* (on a logarithmic scale) for each site at which >10 specimens were collected (Supplementary Table S1): (a) Asinara (Site 10), (b) Zembra archipelago (data pooled for Sites 51–55), (c) Capo Testa (25), (d) Galeria (1), (e) Kelibia (45), (f) El Haouaria (44), (g) Porto Rafael (24), (h) Olbia (18).

TABLE 2 One-way ANOVA for the differences in size between populations of *P. ferruginea*. Only the eight populations where >10 individuals were recorded were included in this analysis. Results of the Student–Newman–Keuls test are ordered by mean size from high to low.

Source of variation	SS	df	MS	F	P
Between populations	357.70	7	51.10	11,156	<0.001
Within populations	4,672.01	1,020	4.58		
Total	5,029.71	1,027			

Student–Newman–Keuls test: Asinara > Capo Testa = Zembra = Galeria = Porto Rafael = Olbia = Kelibia = El Haouaria.

Multivariate analyses indicated differences in population structure with the level of exploitation. Populations in areas free of human visitation (protected) partially segregate from the human-affected sites in the MDS analysis, mainly

appearing in the upper half of the graph (Fig. 3). SIMPROF analysis showed three clusters: one of small populations at frequently visited sites (less protected) on Corsica, Sardinia and mainland Tunisia, a second of mainly protected sites (both on natural and artificial substrates), and a third of several North African populations from the Strait of Gibraltar to the Zembra archipelago in the Siculo-Tunisian strait. PERMANOVA analysis indicated that protection (as a result of inaccessibility) influenced the size frequency distribution of the populations, whereas the type of substrate (natural vs artificial) had no significant influence on population structure (Table 3).

Discussion

The main conclusion of this study is the vulnerable situation of *P. ferruginea*. Although previous studies identified the

TABLE 3 PERMANOVA analysis of the effect of substrate and protection on the size (measured on the longitudinal axis) structure of *P. ferruginea* populations. The matrix used was the same as for the MDS analysis (Fig. 3).

Source	Df	SS	MS	Pseudo- <i>F</i>	P
Substrate	1	421.56	421.56	0.36	0.786
Protection	1	3,516.30	3,516.30	2.96	0.042
Substrate × protection	1	29.90	29.90	0.03	0.994
Residual	17	20,164	1,186.10		
Total	20	24,111			

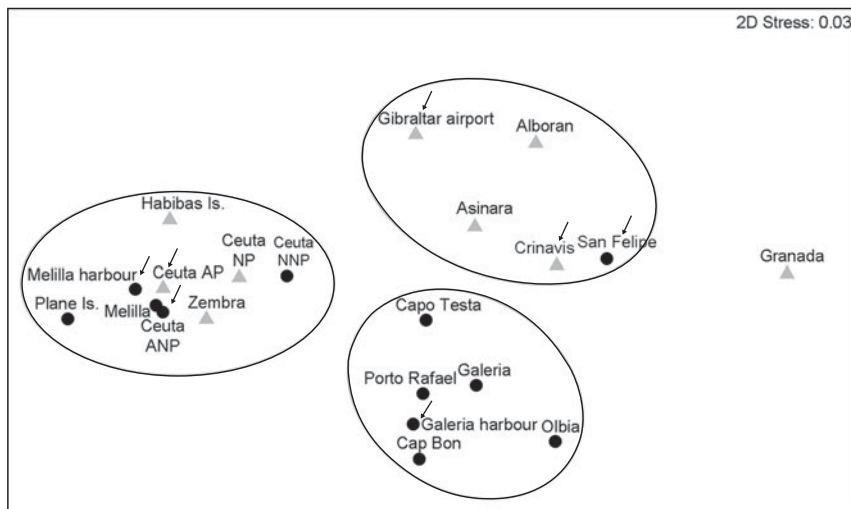


FIG. 3 MDS ordination of populations (only those with >10 individuals) of *P. ferruginea* based on the number of individuals per size class. Groups of samples determined by the SIMPROF test are enclosed by ellipses. Populations include six from this study (Table 2) and 15 from published data (Table 4). Designation as unprotected (circles) or protected (triangles) indicates the likelihood or not, respectively, of exploitation, based on accessibility. ANP, artificial substrate, non-protected; AP, artificial substrate, protected; NNP, natural substrate, non-protected; NP, natural substrate, protected. Arrows indicate artificial substrates.

threatened status of the species (Paracuellos et al., 2003; Bazairi et al., 2004; Guerra-García et al., 2004; Espinosa, 2006; Cristo & Caronni, 2008; Espinosa et al., 2009a,b, and references therein), mainly a result of human pressure, these studies were at a local or regional scale. The information summarized here demonstrates that the range of this threatened mollusc has contracted severely and that the remaining populations are scarce and generally sparse.

The species has not been reported from the Italian mainland since the 1980s, when it was recorded in low numbers at a few locations in Tuscany (Curini-Galletti, 1979; Terreni, 1981; Biagi & Poli, 1986) and one location in Liguria (Porcheddu & Milella, 1991). However, we did not find the species at any of these locations. The situation on the Italian islands of Egadi, Sicily and Pantelleria is also critical. Giaccone & Sortino (1974) reported *P. ferruginea* on the Egadi islands with a relative abundance of II, within a range from I (rare) to V (abundant), but it is now extremely scarce, having also declined in density. Giaccone et al. (1973) reported the species in a general phytobenthos study at Pantelleria but we detected only one small specimen there, despite the focus on this species. There are some specimens from Palermo, Sicily, in Locard's 1892 collection at the Muséum national d'Histoire naturelle, Paris, but the species no longer occurs on the island.

The Corsican and Sardinian populations were greater in density than those on the Egadi islands and Pantelleria but

there has also been a noticeable decline on these islands, with a large decrease in mean density at several sites from 1985 to 2009: 0.933 to 0.02 m⁻¹ at Tizzano (Laborel-Deguen & Laborel, 1990; present study) and 2.334 to 0.13 m⁻¹ at Gáléria (Laborel-Deguen & Laborel, 1991b; present study; Fig. 4). Exploitation in Corsica during summer has been reported by Laborel-Deguen & Laborel (1990, 1991b).

In Tunisia *P. ferruginea* is restricted to the north-eastern shores, as also reported by Tlig-Zouari et al. (2010). The largest and most structured population (i.e. presence of different size classes) is on the Zembra archipelago, although Tlig-Zouari et al. (2010) reported lower densities at Zembra than at Cap Bon. However, on Zembra they surveyed at a location with the lowest densities, which is probably not representative of the archipelago. The species shows great variability in recruitment (Guallart et al., 2006; Rivera-Ingraham, 2010) and the high densities found at some sites at Cap Bon by Tlig-Zouari et al. (2010) could be a result of good recruitment from the Zembra population during 2006.

Many authors have demonstrated the influence of exploitation on the mean size and presence of large reproductive individual molluscs (Keough et al., 1993; Branch & Odendaal, 2003). *Patella ferruginea* is used as food and fishing bait and as a collector's item (Laborel-Deguen & Laborel, 1991a, b; Templado & Moreno, 1997; Ramos, 1998), the latter confirmed by our observations of shells being sold

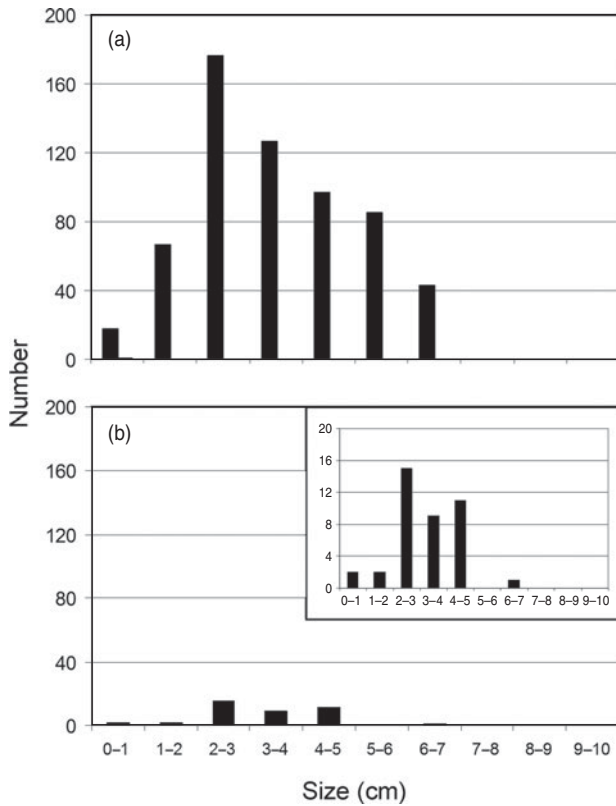


FIG. 4 Size (measured on the longitudinal axis) frequency distribution of *P. ferruginea* at Galéria, Corsica, in (a) 1985 (data from Laborel-Deguen & Laborel, 1991b) and (b) 2009 (present study).

as souvenirs at the tourist site of Kerkouan, Carthage. Accessibility is the most important factor influencing collection, regardless of the legally protected status of the species (Guerra-García et al., 2004; Espinosa et al., 2009a). To protect threatened molluscs such as *P. ferruginea* we recommend, based on our findings, use of marine protected areas combined with effective control of human visitation, as has been implemented on Asinara island and the Zembra archipelago. Although exploitation is evident in several areas of Sardinia (Cristo & Caronni, 2008), the marine protected area of Asinara has the largest individuals and highest densities in the Corsica–Sardinia region, and the population in the Zembra marine protected area (established in 1977 and where human visitation is prohibited) showed strong recruitment and had many large reproductive individuals, indicating a well-structured and viable population (Espinosa et al., 2009a). Changes in the population on Zembra during the last 25 years have been positive (Fig. 5) in terms of density (from 0.7 m⁻¹ in 1986 to 2.65 m⁻¹ in 2009) and mean length (mean of specimens >2 cm was 4.4 cm in 1986 and 5.4 cm in 2009). Marine protected areas (Asinara, Zembra: present study; Habibas and Chafarinas islands: Espinosa, 2009; Guallart et al., 2006) and also non-legally protected sites, many of them on artificial substrates

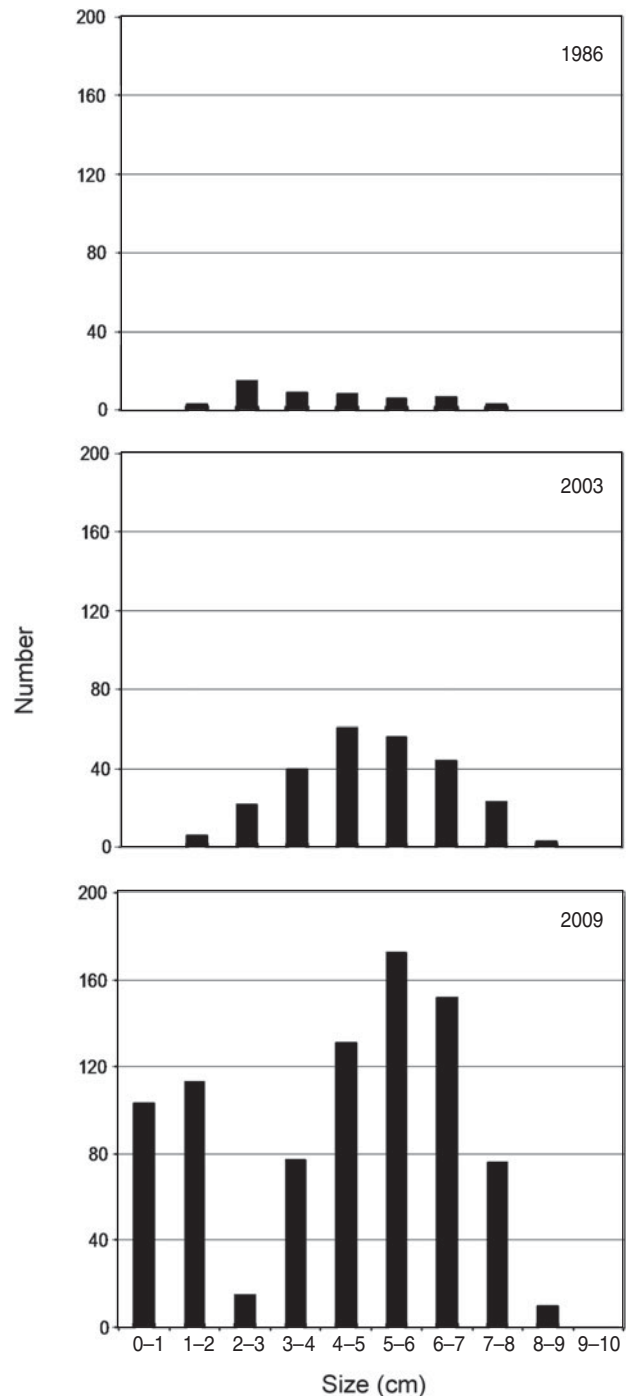


FIG. 5 Size (measured on the longitudinal axis) frequency distribution of *P. ferruginea* at Zembra in 1986 (adapted from Boudouresque & Laborel-Deguen, 1986), 2003 (adapted from Limam et al., 2004) and 2009 (this study).

(e.g. Algeciras Bay and Ceuta; Espinosa et al., 2009a), could be a source of larvae for adjacent areas. Connectivity between populations needs to be guaranteed by establishment of a large number of small and proximate (no more than 10–20 km apart) marine protected areas, as suggested by Boudouresque et al., (2005).

TABLE 4 Summary of previous reports of the presence of *P. ferruginea*. Map numbers correspond to numbered locations in Fig. 6.

Map no.	Location	Source	Density (m^{-1})	Estimated total population
1	Port Cros (France)	Cottalorda et al. (2004)	<0.01	2
2	Hormigas Island (Spain)	Espinosa et al. (2009b)	<0.01	2
3	Andalucía (Spain)	Barba et al. (2006)	<0.01	715 (1,005)*
4	Algeciras Bay (Spain)	Espinosa et al. (2005)	0.11	140
5	Ceuta (Spain)	Rivera-Ingraham (2010)	2.26	43,992
6	Alboran Island (Spain)	Paracuellos et al. (2003)	0.07	111
7	Al Hoceima (Morocco)	Bazairi et al. (2004)	0.24	110
8	Melilla (Spain)	González-García et al. (2006)	5.39	20,000
9	Chafarinas Island (Spain)	Guallart et al. (2006)	3.95	50,000
10	Habibas Island (Algeria)	Espinosa (2009)	4.8	50,400
11	Plane Island (Algeria)	Espinosa (2009)	22	?
12	Stidia (Algeria)	Mezali (2005)	9.75	?
13	Srigina Island (Algeria)	F. Bernard (pers. comm.)	<0.01	?
14	Lavezzi Island (France, Italy)	Mari et al. (1998)	0.1	1,000
	Zembra (Tunisia)	This study	2.65	34,450

*Value from the most recent survey (Junta de Andalucía, 2010).

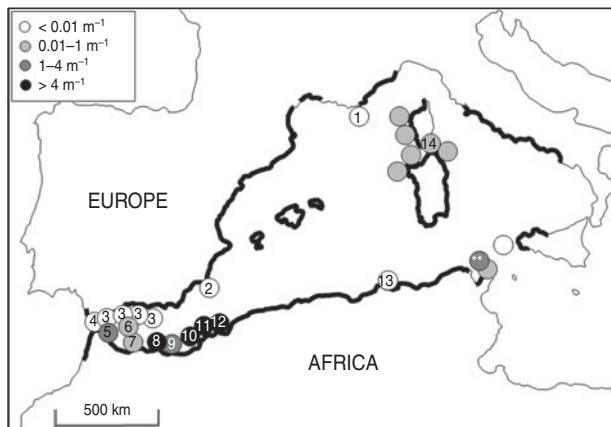


FIG. 6 Present locations (circles) of *P. ferruginea* based on recent reports and the results of our survey. Shading of circles represents density. Numbers in the circles correspond to the map numbers in Table 4; unnumbered circles indicate values obtained in this study (Table 1). Thicker lines along the coast indicate the range of the species in the 19th century (Laborel-Deguen & Laborel, 1991a).

The distribution of *P. ferruginea* in the western Mediterranean is highly fragmented (Fig. 6). The status of the species is critical in continental France, where some individuals survive in the Port Cros marine protected area (Table 4; Cottalorda et al., 2004), probably derived from Corsican populations via the marine currents that flow westward from Corsica to the Gulf of Lyon (Robinson et al., 2001). The species is absent from the Mediterranean shores of the Iberian Peninsula (from the Gulf of Lyon to the Hormigas Islands; Espinosa et al., 2009b), with the exception of some relict populations in Andalusia, mainly on the shores of Granada and Algeciras Bay (Espinosa et al., 2005; Barba et al., 2006). The species was not considered rare in Algeciras Bay by García-Gómez (1983), and Grandfils

(1982) reported small populations along the promenades of Malaga and Fuengirola (Andalucía). However, recent data indicate that the species is scarce in the area and has disappeared from some sites. The estimated total number of individuals in the Iberian Peninsula is only c. 1,000 (Table 4). However, North African populations from the Strait of Gibraltar to Algeria exhibit high densities. The main populations are at Ceuta, Melilla, Chafarinas and the Habibas Islands (Table 4). Several sites have also been recorded in Mediterranean Morocco (from near Ceuta to close to the Chafarinas Islands): Midiaq, Restinga, Cabo Negro, Oued Laou, El Jahba, Cabo Tres Forcas, Cabo del Agua and Essaidia (Bazairi & Benhissoune, 2004; Bazairi et al., 2004; Espinosa, 2006; Bazairi & Benhissoune, 2007). Of these, the population in Al Hoceima National Park is important from a conservation perspective (Bazairi et al., 2004). The populations in western Algeria (Rachgoun and Habibas islands) exhibit high densities, a well-structured size distribution and high reproductive output (Frenkiel, 1975; Boumaza & Semroud, 2001; Espinosa, 2009). There is a lack of data from western Algeria to the Tunisian border, although isolated observations (very low density on Srigina island, Table 4; F. Bernard pers. comm.) indicate there are no significant populations in this area, consistent with the species' absence along the northern shores of Tunisia (Tlig-Zouari et al., 2010; present study). Thus the range of the species in the western Mediterranean has contracted since the 19th century (Fig. 6). For example, Payraudeau (1826) reported *P. ferruginea* as very common in Corsica but it is now rare.

Although *P. ferruginea* is listed under European regulations and those of several Mediterranean countries it has not previously been assessed for the IUCN Red List. It could be categorized as Critically Endangered based on criteria A and E (IUCN, 2012). A population viability analysis (PVA)

for the species indicated a decrease of >80% in the next 10 years (criterion A3) if the present collection rates continue at harvested sites (without larvae supply from well-preserved populations; Rivera-Ingraham et al., 2011), and many populations of *P. ferruginea* have shown a decrease of >80% in the last 10 years (criterion A2), as reported by our study, including the populations of the southern Iberian Peninsula, Corsica, Italy, Egadi and Pantelleria islands and the Tunisian mainland. The PVA by Rivera-Ingraham et al. (2011) showed an extinction probability of >50% in the same period (criterion E). Their analysis was carried out on one of the most important relict populations (in Ceuta), and any PVA analysis of other small populations would probably reveal a greater extinction probability.

The fact that *P. ferruginea* occurs on artificial substrates throughout the western Mediterranean (Espinosa et al., 2009a; García-Gómez et al., 2011) is critical when evaluating the potential threats to the species. When separated from demographic functionality, assessments of density may lead to incorrect conclusions about the value of artificial structures as alternative habitats because they may act only as ecological sinks in which organisms fail to breed successfully (Smallwood, 2001). However, our study indicates that exploitation is the main factor influencing population structure and, consequently, potential population viability, and the type of substrate has no significant influence. In this context, a proposal for artificial marine micro reserves (García-Gómez et al., 2011) could be an effective conservation measure for *P. ferruginea*, protecting the relatively small areas of artificial substrates on which the species has settled. However, further studies are required to elucidate the population dynamics and ecological functioning of *P. ferruginea* populations on both artificial and natural substrates.

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Biographical sketches

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