POPULATION DYNAMICS AND VIABILITY ANALYSIS FOR THE CRITICALLY ENDANGERED FERRUGINEAN LIMPET

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ABSTRACT The current study deals with the critically endangered limpet *Patella ferruginea* (Gastropoda: Patellidae) endemic to the western Mediterranean. The species has been in decline since the early 20th century and is currently restricted to certain locations on the Iberian Peninsula, Corsica, Sardinia, and the North African coasts of Morocco, Algeria, and Tunisia. Its large size and conspicuous shell often makes the species a target of human collection. We describe the results of temporal monitoring conducted on one of the remaining most important *P. ferruginea* populations in North Africa, and provide quantitative data on growth rates, natural mortality, and harvesting rates. The maximum collection rates were recorded during the summer months, when fishermen most attend the beach. This type of mortality mostly affected medium and large individuals, and increased natural mortality rates up to 37%. All results and previously available data were implemented for population viability analysis. We determined that the species is clearly overexploited in the study area, and may face local extinction within the next 20 y if harvesting activities are not controlled. Even though more precise predictions could be obtained by using a longer time series, our study is the first attempt to model the future viability of the species, and indicates the urgent need of establishing efficient protection measures.

KEY WORDS: conservation, endangered species, limpet, *Patella ferruginea*, population viability analysis, PVA

INTRODUCTION

Population viability analysis (PVA) is a group of mathematical tools that allows predicting extinction probability for a certain species, considering all factors that affect the processes of species extinction (Gilpin & Soulé 1986) and how environmental and ecological factors affect birth, death, and immigration and emigration rates over time. These PVAs have been used successfully to test the effect of conservation measures on the future viability of populations (see the review by Miller (2003)). A wide variety of analyses can be carried out depending of the complexity of the initial model, from the simplest (which only require count data) to more complex ones (which treat each specimen individually and its relation with other specimens and its habitat). Other more complex models require the estimation of substantially more parameters. However, when dealing with endangered species, the available information is frequently scarce and incomplete, and because of the species' status, experimental studies are not always possible. This is why the use of simple PVAs is starting to be recommended more frequently (Beissinger & Westphal 1998, Holmes 2001, Morris et al. 2002). In any case, to use these tools, time series data are required to estimate basic parameters such as population growth, natural mortality, harvesting rates, and so forth.

The current study deals with *Patella ferruginea* Gmelin, 1791 (Gastropoda: Patellidae) (Plate 1), which is a giant intertidal limpet (reaching up to 10.7 cm) (Rivera-Ingraham 2010) endemic to the western Mediterranean basin. It is considered to be the most endangered marine invertebrate species on the list of the European Council Directive 92/43/EEC, as populations have suffered clear regression since the beginning of the

20th century (Laborel-Deguen & Laborel 1991, Templado 2001). Currently, populations are mainly distributed along the North African coasts, being distributed from Ceuta (Strait of Gibraltar) (Guerra-García et al. 2004, Espinosa 2006, Espinosa et al. 2009a; Rivera-Ingraham et al. 2011b) to Cape Bon and Zembra Island (Tunisia) (Boudouresque & Laborel-Deguen 1986, Espinosa 2006). However, European coasts are home to small and scarce populations in southern Spain (Espinosa et al. 2005; Moreno & Arroyo 2008), Italy (Curini-Galletti 1979, Biagi & Poli 1986, Porcheddu & Milella 1991, Doneddu & Manunza 1992, Cristo et al. 2007, Cristo & Caronni 2008), and France (Corsica) (Laborel-Deguen & Laborel 1991).

Most of the PVAs conducted to date have focused on vertebrates, whereas invertebrate species (most of them insects) have only been the subject of some studies (e.g., Murphy et al. 1990, Schultz & Hammond 2003, Bergman & Kindvall 2004). However, only a very small proportion of the latter consider marine invertebrates (e.g., Linares et al. 2007). The objective of the current study is to obtain basic biological data (e.g., growth rates, natural mortality, and harvesting rates) for *P. ferruginea* through temporal monitoring of populations, and to use these data to carry out PVA, and to predict extinction risks and propose adequate conservation measures for the species.

MATERIALS AND METHODS

In the current study, Ceuta is considered to host a *P. ferruginea* metapopulation, which would be composed of a certain number of identical subpopulations genetically interconnected (Levins (1969) and Levins (1970) cited in Hanski (1999)).

Study Site

The current study was conducted on the North African coasts of the Strait of Gibraltar (Fig. 1A). Our study area is Ceuta (Fig. 1B), where one of the remaining most important *P. ferruginea*

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Plate 1.

populations (constituted by approximately 44,000 individuals) (Rivera-Ingraham et al 2011b) can be found. It is well known that the consideration of different type of populations (subject of diverse rates/intensities of impact) can help us test different working hypotheses simultaneously (Francis & Hare 1994). In consequence, a total of seven of the most important subpopulations in Ceuta (in terms of density) were used to carry out the temporal analyses of populations, located on both the North and South Bays, on both natural and artificial substrates and presenting different degrees of impact by recollection (harvesting rates; Table 1).

Temporal Analysis of Populations

Between August and September 2007, a 100-m area was delimited in each of the populations considered (Table 1). Each of these areas was subdivided into three 10-m transects (T1, T2, and T3), separated from each other by 35 m. The limits of the areas and transects were marked using red paint, and there coordinates were registered using a GPS Garmin MAP76 (Garmin Ltd., Olathe, KS) in case the red paint faded through time.

Taking into account that *P. ferruginea* is a protandric species, individuals were classified in three size classes based on their maximum shell size (roughly corresponding to the different sexual stages): less than 25 mm (sexually immature individuals), 25–50 mm (primarily composed by male individuals), and more than 50 mm (mainly females) (Espinosa et al. 2009b). In each transect, a total of 30 individuals (10 per size class), when possible, were marked using an epoxy bicomponent resin (Eporai 1127), over which an individual identification number was stamped before the material dried. To avoid false mortality recording resulting from the loss of shell tags, control tags (with the same ID number) were established on the substrate next to the individual's home scar. The presence of a tag next to an empty scar indicates the death of the studied individual.

The temporal analysis of the populations was carried out between 2007 and 2009. This was done every 3 mo, considering both the previously marked individuals and the total number of specimens in the area. Regarding the first, the maximum shell length (recorded to the nearest millimeter using calipers) was registered for each tagged individual. Any lost tags were re-

Figure 1. (A) Southwestern Europe. The dotted circle indicates the study site's location (Ceuta), on the North African coast of the Strait of Gibraltar. (B) Ceuta, where a total of 7 important *Patella ferruginea* subpopulations were selected to conduct the study. a, Chorrillo; b, Foso de San Felipe; c, Sarchal; d, Desnarigado; e, Dique de Levante; f, Parque del Mediterráneo; g, Benítez.

established. When less than 10 individuals per size class were marked (because of mortality or growth of individuals), more individuals were marked to maintain the initial conditions of the experiment. At the same time, censuses were carried out. During the first year of the study, the 100-m area delimited for each population was completely counted. However, because of the large amount of effort and time required to complete this task, during the second year of the study, only the 10-m transects were counted.

To determine microalgal availability throughout the year, the temporal analysis of populations also included the collection of 5 rock chips in each area, which were obtained from the *P. ferruginea* tidal height with the help of a hammer and chisel. These rock samples were fixed in methanol and preserved in dark, cold conditions (4°C) for a minimum of 48 h to allow for pigment extraction. This solvent was selected based on the results provided by Nagarkar and Williams (1997), who determined that methanol and ethanol are more efficient than acetone for extractions, even without having to heat the solvent or homogenize the sample (Holm-Hansen & Riemann 1978). Each rock chip was photographed along with a millimeter scale, and the resulting images were exported to Scion Image Alpha 4.0.3.2

TABLE 1.
General characteristics for each of the populations considered in the study.

Site	Adult Density (ind./m)	Overall Density (ind./m)	Substrate	Harvest Rate	Maximum Size (cm)	Location
Chorrillo	6.19	6.86	Artificial	High	8.60	South Bay
Foso	2.05	4.31	Artificial	High	8.70	South Bay
Sarchal	0.86	1.04	Natural	High	6.40	South Bay
Desnarigado	2.31	2.97	Natural	Medium	7.50	South Bay
Dique Levante	4.55	7.09	Artificial	Low	7.80	North Bay
Parque del Mediterráneo	5.39	6.81	Artificial	Low	10.00	North Bay
Benítez	1.76	2.73	Artificial	Medium	6.60	North Bay

Numbers associated with the site name are correlated with those described in Figure 1. ind., individual.

(2000–2001 Scion Corporation, Frederick, MD) to calculate the rock chip's area. The solvent was filtered using a Whatman GF/C filter. Absorbance was determined by using a Zuzi 4110 ED spectrophotometer. Results were expressed in terms of micrograms chlorophyll per square centimeter by using the following equation (Thompson et al. 1999):

$$\text{Chlorophyll} = 13.0 \times A_{665} \times [V/(d \times A)]$$

where 13.0 is the constant for methanol, A_{665} is the sample's absorbance at 665 nm, V is the sample's final volume, d is the path length of the cell, and A is the chip area.

The PVA Model

Analyses were carried out using RAMAS EcoLab (1990, Applied Biomathematics, Setauket, NY). A simple matrix model was selected to conduct the PVA, using the results obtained during the current study as well as previously available data. As mentioned earlier, individuals were classified into three different size classes based on their maximum shell length. Both mean and SD values were calculated for (1) natural surviving rates (NSR), calculated using the total number of individuals marked in Parque del Mediterráneo, which was the only area clearly presenting null impact of harvesting because it is completely inaccessible because it is private property; (2) annual mortality rate (AMR), calculated using the total number of tagged individuals (except the ones in Parque del Mediterráneo); (3) average harvesting rates, calculated as $\text{AMR} - (1 - \text{NSR})$ for each area; and (4) population growth (PG), calculated as the interannual variation of the total number of individuals found in the 10-m transects ($\text{PG} = \text{Nt}_n/\text{Nt}_{n+1}$).

Other parameters were also included in the initial model, including total fecundity, which was considered as the mean oocyte production per female and size class. To estimate this parameter, the probability of finding a female for each size class was determined first. This was done by using the latest available data regarding sex distribution for each of the considered subpopulations provided by Rivera-Ingraham et al. (2011c), and by knowing the size and total number of individuals in each area. Consequently, a number of individuals (equivalent to the previously calculated percentage) were randomly selected to estimate oocyte production. This was done using the data provided by Espinosa et al. (2006) to avoid sacrificing an unnecessary high number of individuals. Espinosa et al. (2006) established that for *P. ferruginea*, the individual's maximum

shell length in centimeters (x) was correlated with the gonad weight in grams (y) by the following equation: $y = 0.000216 \times x^{5.08}$. By using this formula, the estimated female gonad weight for each of the randomly selected individuals was calculated. Finally, and to determine the total number of oocytes present in the gonads, we again used the data provided by Espinosa et al. (2006), who established that 1 mg of female *P. ferruginea* gonad represents an average of 302 oocytes. When the number of oocytes was calculated for each of the individuals previously selected as females, the average fecundity values per size class were obtained. However, these values need to be expressed as a percentage. The obtained values were transformed by considering the oocytes produced by the largest sexed female (10 cm) as 1 (100%), and referring the rest of the values accordingly.

Another parameter included in the initial model was estimation of the total number of individuals (per size class) in the metapopulation. The latest estimation of the total number of *P. ferruginea* individuals indicates that Ceuta may contain ~44,000 individuals (Rivera-Ingraham 2010). Based on the first complete census carried out in the context of the current study (November 2007), the percentage of individuals representing each size class was calculated and then extrapolated to the total number of individuals that would comprise the metapopulation, and the total number of individuals present in Ceuta per size class was calculated for $t = 0$ (beginning of the experiment).

The third parameter included in the initial model was the stage average weight. RAMAS software considers one unit of time as the number of years passing between reproduction events. In this sense, individuals would require one of these units of time to pass from one size class (or age class) to the next one. In the case of *P. ferruginea*, the time needed for an individual to pass from one size class to the other is not constant, because growth rate depends on the age of the individual (Espinosa et al. 2008). To make the necessary corrections to the model, the annual growth rates for individuals initially belonging to the different size classes considered were calculated for both the first year (2007 to 2008) and the second year (2008 to 2009) of study. Later, the time needed for an individual to reach a size of $x + 25$ mm (and pass to the next size class) was calculated, and was considered as the stage's average weight.

Regarding the simulation's conditions, the demographic stochasticity option was used, which allows us to consider the

existence of variations in the annual recruitment, and growth and mortality rates, even when environmental conditions are stable. In addition, RAMAS also enables us to select the type of relationship between density and time. For the current study, the “scramble” model was chosen, taking into account that *P. ferruginea* is not a territorial species and that it is present in intertidal levels, where trophic resources are limited. Associated with this selection, carrying capacity needed to be established. Because there are no available data regarding this value, we used one of the highest density values registered in Ceuta (6.86 individuals/m) (refer to Rivera-Ingraham (2010)). Considering that Ceuta presents around 19 km of rocky substrate where the species could potentially be present, we estimated that the metapopulation's carrying capacity could be established at 100,000 individuals.

After the software was configured with the aforementioned parameters, we ran simulations with 1,000 replications and for a time period of 50 y.

Statistical Analyses

For both parametric and nonparametric analyses, the SPSS 15.0 (SPSS Inc. Chicago, IL) statistical package was used.

RESULTS

Evolution of Population Density and Recruitment

A strong seasonal component in the frequency of individuals is revealed when observing the results from the censuses of the three 10-m transect in each area (from 2007 to 2009; Fig. 2). Those areas almost or completely protected from human collection (sites 5 and 6) show an increase in the number of individuals (for all size classes considered) throughout the duration of the study. On the contrary, in those areas easily accessible by humans (such as sites 1, 3, and 7), medium and large individuals (>25 mm) considerably decrease during the summer and early autumn months. In any case, all areas showed an increase in the number of recruits (<25 mm) in spring and summer months. Comparing these results with those obtained from counting the entire 100-m area, we observed no important differences in terms of the evolution of the figures' profiles.

Recruitment quantification was especially easy by using the population size structures recorded through censuses (Fig. 3). Through cohort analysis, 2 recruitment events were detected during the study (1 event/y). The difference between these two events was especially noticeable, with the second (2009) producing 335 more recruits (+41.2%) than the first (2008). Figure 3 also helps visualize recruit growth rate patterns through time, and indicates clearly the high values registered during the spring months (discussed later). Asynchronous recruitment through time is also evident. Pearson's test was carried out to search for any correlations between the number of recruits registered during summer months and the most common population parameters. Results indicate that this parameter is correlated with total number of adults (>25 mm) located in the same transect. Eleven different regression models were performed through curve estimation regression analysis, and a linear model provided the optimal fit, presenting the best adjustment ($R = 0.932$, $P < 0.001$). Those areas presenting a lower number of adults also presented lower recruitment rates.

Growth Rates

For the calculation of growth rates, Gulland and Holt's (1959) equation was used for marked individuals. This type of monitoring (using individual tags) was especially useful for the registration of growth rates in medium and large individuals.

The annual growth rate of individuals was negatively correlated with individual's shell size. Smaller individuals presented the highest growth rates (1.39 ± 0.67 cm)—significantly higher than medium (1.09 ± 0.65 cm) and large individuals (0.50 ± 0.39 cm; chi-square value = 50.30, $P = 0.000$). Growth rates also presented a clear seasonal component. For all size classes, individuals showed the lowest growth rates during autumn months, and a maximum during summer months (Fig. 4). Moreover, and also for all size classes considered, average growth rate values were significantly higher in populations located on Ceuta's South Bay (Table 2).

Natural Mortality Rates

As mentioned, to calculate the natural mortality rates for *P. ferruginea*, the Parque del Mediterráneo was the only area taken into consideration (where null human harvesting rates can be ensured). Monthly mortality rates did not present statistically significant values between size classes ($F = 2.29$, $P = 0.126$). The recruit fraction of the population (<25 mm) showed an average monthly mortality rate of $0.28 \pm 0.57\%$. The medium and large size fractions of the population (25–50 mm and >50 mm) showed $3.42 \pm 2.28\%$ and $3.41 \pm 5.37\%$ average monthly mortality rates, respectively. No significant differences were registered in natural mortality rates throughout the year either ($F = 1.01$, $P = 0.410$). In any case, winter months showed slightly lower monthly values ($0.38 \pm 0.93\%$), lower than the autumn ($3.14 \pm 3.90\%$), spring ($3.72 \pm 5.12\%$), and summer ($2.23 \pm 2.92\%$) months.

Human Collection (Harvesting) Rates

The lowest annual harvesting rates were recorded for the smallest individuals (<25 mm; $2.46 \pm 3.47\%$). Annual rates of $12.15 \pm 15.82\%$ and 26.24 ± 20.14 were recorded for medium and large individuals, respectively. Moreover, these presented an important seasonal component, with the winter months having the lowest harvesting rates (Table 3).

Population Viability Analysis

RAMAS simulations predicted that the *P. ferruginea* metapopulation in Ceuta is clearly overexploited. The total number of individuals in the area will decrease considerably during the next decades if harvesting rates continue constant for 50 y. Ceuta's metapopulation would be left with an average of 5,707 individuals (a reduction of 87%) and 923 individuals (97.9% reduction) in 10 y and 20 y, respectively (Fig. 5A). These values would be valid if all populations present in Ceuta were subject to collection, however, we can find some inaccessible areas where no harvesting evidence has been registered (e.g. Parque del Mediterráneo). If these type of populations cannot supply the rest with enough larvae and if collection continues, the species could face local extinction in 20 y.

Supposing that the species is completely free from collection (because of effective protection measures), a new simulation (with the same parameters but considering only natural mortality rates) predicted that Ceuta's metapopulation would significantly

Figure 2. (A–G) Evolution of the total number of individuals in the three 10-m transects through time for the 2-y duration of the study. Letters correspond to each of the sectors considered (refer to Fig. 1). Continuous lines and rhombuses correspond to the number of individuals smaller than 25 mm. Discontinuous lines and squares represent values for individuals from 25–50 mm maximum shell size. Dotted lines and triangles represent values for individuals larger than 50 mm.

increase the number of individuals, up to an average of 66,923 (+52%) and 80,252 (+82%) individuals in 20 y and 50 y, respectively (Fig. 5B). On the other hand, the species' 50% probability of extinction is established at 33,000 y. Yet another

simulation, considering only the protection of individuals larger than 50 mm, predicts that the metapopulation would still increase up to an average of 53,094 and 60,506 individuals in 20 y and 50 y, respectively (Fig. 5C), which is a difference of only 31% and 44%

Figure 3. Total number of individuals registered in the 10-m transects per size (classified in 0.5-cm intervals) and time. Dotted curves indicate recruit cohorts.

less increase than if the complete population was effectively protected. Under this new situation, the species' 50% probability of extinction is established at 29,000 y.

DISCUSSION

Temporal series can be considered of great importance when dealing with endangered species. It is essential to know basic biological aspects of the species under consideration to propose adequate conservation measures. Throughout 2007 and 2009, we obtained valuable information regarding some of these parameters.

Evolution of Population Density and Mortality Rates

The evolution of the population structure and the mortality rates were clearly dependant on the area's accessibility to humans. It has already been determined that the area's accessibility can determine the population's size structures for intertidal invertebrates (e.g., Kido & Murray 2003, Espinosa et al. 2009a). Inaccessible areas like Parque del Mediterráneo presented similar natural mortality rates for all size classes considered and through time. This is in agreement with the results obtained by Espinosa

et al. (2008). However, this is an expected result in light of the bet-hedging interpretation of life history patterns, which predicts higher mortality rates for juveniles than adults in long-lived invertebrate species (Stearns 1992) such as *P. ferruginea*. But for those areas commonly frequented by fishermen, mortality rates were higher for medium and large individuals, and clearly presented a seasonal component. *P. ferruginea* individuals present eye-catching shell morphology, and when individuals reach medium and large shell sizes they become very conspicuous and, in consequence, these fractions of the populations are frequently subject to collection (Guerra-García et al. 2004, Espinosa 2006, Espinosa et al. 2009a), as happens with other giant limpets (Keough et al. 1993, Lasiak 1993, Branch & Odendaal 2003, Sagarin et al. 2007). We observed that accessible areas were highly frequented by fishermen (except during winter months). These fishermen usually collect intertidal organisms such as limpets for food, as fishing bait, or simply for ornamental purposes (e.g., Laborel-Deguen & Laborel 1991, Keough et al. 1993, Ramos 1998, Kido & Murray 2003). In easily accessible areas, the largest (and most conspicuous) individuals showed the greatest interannual density variations, always associated with fair-weather months, when fishermen most attend the beach. In some of the

Figure 4. Average growth values per size class and season. Bars indicate SEs. Groups associated with the same letter and color belong to the same season subset based on subsequent Mann-Whitney U-tests.

study areas, the largest fraction of the population has even disappeared (e.g., Sarchal). All this evidence and observation, along with the simulations provided by the PVA, allows us to conclude that collection rates are highly influencing the evolution and viability of Ceuta's metapopulation, as discussed in detail later.

Recruitment

The presence of recruits started to be evident in the spring and through the summer (up to August and September), which is supported by the observations made by Espinosa (2006) and Rivera-Ingraham (2010). These authors suggest the possibility that, after gamete fecundation, and after settlement, individuals grow during this period of time until they are able to be detected during the summer surveys. However, it has been observed that *P. ferruginea* individuals may release gonad contents throughout long-term periods (finding, in Ceuta, sexually mature individuals from September to early February) (Rivera-Ingraham 2010), and, in consequence, produce the asynchronous appearance of recruits observed here.

TABLE 2.

Mann-Whitney U-test results for the influence of the population's location on the average growth rate for each size class considered.

Source of Variation	Mean $\pm$ SD (mm/month)	n	Z Value	P Value
(1) Location			-4.55	***
North Bay	1.07 $\pm$ 0.90	287		
South Bay	2.04 $\pm$ 2.24	263		
(2) Location			-11.48	***
North Bay	0.80 $\pm$ 0.90	572		
South Bay	1.73 $\pm$ 1.69	589		
(3) Location			-7.40	***
North Bay	0.47 $\pm$ 0.61	217		
South Bay	1.07 $\pm$ 1.10	288		

*** $P < 0.001$.

(1) Individuals < 25 mm. (2) Individuals 25–50 mm. (3) Individuals > 50 mm.

TABLE 3.

Kruskal-Wallis nonparametric test results for the influence of season on the monthly harvesting rates for each size class considered.

Source of Variation	Mean $\pm$ SD (%)	df	Chi-square Value	P Value
(1) Season		3	5.47	NS
Autumn	2.49 $\pm$ 3.99			
Winter	4.79 $\pm$ 9.25			
Spring	0.20 $\pm$ 0.62			
Summer	4.32 $\pm$ 4.94			
(2) Season		3	17.16	***
Autumn	6.96 $\pm$ 6.63 ^a			
Winter	0.78 $\pm$ 1.22 ^b			
Spring	4.44 $\pm$ 7.12 ^a			
Summer	10.96 $\pm$ 9.99 ^c			
(3) Season		3	10.56	*
Autumn	4.89 $\pm$ 4.06 ^a			
Winter	1.82 $\pm$ 4.05 ^b			
Spring	10.08 $\pm$ 8.53 ^a			
Summer	3.74 $\pm$ 6.53 ^a			

* $P < 0.05$.

*** $P < 0.001$.

(1) Individuals less than 25 mm. (2) Individuals 25–50 mm. (3) Individuals > 50 mm. Values followed by the same letter belong to the same subset based on subsequent Mann-Whitney U-tests.

NS, nonsignificant.

Although previous authors have not been able to correlate recruitment intensity and adult density (Templado et al. 2006), we did detect that the total number of recruits found in the 10-m transects is positively correlated with the number of adult individuals (> 25 mm) present in the same area. This is somehow a predictable pattern, as a higher number of adults will be producing a greater number of gametes and, consequently, a greater number of larvae. However, this could also be an indication that (1) larvae are not being widely distributed and that fecundation and recruitment processes take place locally, or (2) hydrodynamism is indeed influencing larvae distribution, but recruitment would be occurring as a response to larvae attraction by chemical compounds produced by adult conspecifics, as suggested by Rivera-Ingraham et al. (2011), which would explain the aggregation pattern found in the species. This wide distribution of larvae is also supported by the fact that the considered populations are genetically homogeneous (Casu et al. in prep.). It is also interesting to note the important differences in the total number of recruits obtained in the 2 consecutive years studied, having registered 41% more recruits in 2009 than in the previous year. This interannual difference has already been observed in some other important *P. ferruginea* populations (like the one present in Chafarinas Islands) (Templado et al. 2006), and has been explained as differences in the environmental conditions.

Growth Rates

Through the marking of individuals, we were able to study the growth rate patterns in *P. ferruginea*. The use of censuses was especially helpful in dealing with recruits' growth rates, because epoxy tags were difficult to put on such small shells and they would usually end up falling off as a result of wave impact.

Figure 5. Results of the population viability analyses—population trend (1) and extinction probability (2)—for each of the three different situations considered in the study. (A) Population under recorded harvesting rates. (B) Complete and effective protection of the species (considering only natural mortality rates). (C) Complete and effective protection of individuals larger than 5 cm.

In any case, both monitoring methodologies provided valuable information regarding this parameter.

Growth rates in *P. ferruginea* are negatively correlated with the individual's initial size/age, which is the usual shell growth pattern of intertidal gastropods (Frank 1969, Creese 1981). In this sense, recruits present the highest growth rate values, which decrease as we move toward larger shell size, as has already been demonstrated for *P. ferruginea* (Espinosa et al. 2008), *Patella vulgata* (Blackmore 1969) or *Cymbula nigra* (Rivera-Ingraham 2010). Moreover, growth rates presented a clear seasonal component, and all individuals (regardless of size class) presented significantly lower values during the autumn months, and maximum values during the summer months, agreeing with the results previously provided by Laborel-Deguen and Laborel (1991), Espinosa et al. (2008) and Templado et al. (2006). Some authors have pointed out that primary production highly influences growth rates of intertidal gastropods (Underwood 1984), which has been demonstrated for some limpet species (e.g., Hatton 1938, Fischer-Piette 1948). However, our analyses indicate that chlorophyll concentrations were not significantly higher during the summer months. Espinosa (2006) associated these low growth rates with the fact that, during that time, gonad maturation occurs (Frenkiel 1975) and resources may be deviated for gonad development (Orton et al. 1956).

Results also provide evidence of the influence of environmental conditions on growth rates. Individuals located in Ceuta's South Bay (with warmer water temperatures because of the large Mediterranean influence) presented significantly higher growth rates than individuals present in the North Bay (with

colder water temperatures because of the Atlantic influence), and these differences cannot be attributable to differences in chlorophyll concentrations. Based on this and the previously mentioned results, we think that colder water temperatures may be affecting the individual's metabolic rate, as happens with other limpets such as *Nacella magellanica* (Malanga et al. 2007), and producing in consequence lower growth rates.

Considerations Regarding the Monitoring Protocol

Using these two monitoring systems (census and tagging of individuals), basic biological parameters and data regarding the population's evolution were obtained. Based on these observations and results, the following conclusions may be derived:

1. Censuses should concentrate in monitoring small transects instead of large areas, as this considerably reduces the time and effort needed to carry out the census, which could be rather used in monitoring a greater number of populations. When comparing the results obtained from counting a 100-m transect and 3 10-m transects, we determined that no important differences existed.
2. The monitoring of populations should be carried out, when possible, by the same person or team. As it has already been pointed out by others, such as Wienecke et al. (2009), this approach can be considered of great importance to avoid data oscillations resulting from people's ability to detect certain population fractions or to recognize certain individuals.
3. The resin used to create tags should not be an eye-catching color, so that harvesting rates are altered by it.

4. It should be pointed out that many individuals lost their shell tags. This especially affected the smallest fraction of the population (47% of the total recruits marked), which were more easily monitored using a census. In any case, individuals were able to be identified through the control mark established in nearby areas. By using control tags, the over-estimation of mortality rates was avoided.
5. The selection of the populations to be monitored is also important. Different types of populations (presenting different grades of accessibility and density, subject to various grades of collection, and so forth) should be chosen.

Population Viability Analysis

Demographic models have been widely used in population biology for more than 40 y (Levins 1966). Our model was not only based on time series of population abundance, but also on fecundity estimates, growth and natural mortality rates, as well as harvesting rates. In addition, we considered stochastic events in our initial model, which has been considered essential to conservation biology (Shaffer 1981, Sanson et al. 1985, Soulé 1986, Soulé 1987, Simberloff 1988), because these processes can influence population growth and mortality rates (Dennis et al. 1991). Shaffer (1981) was the first to use stochastic models satisfactorily for predicting the evolution of endangered species such as the grizzly bear (*Ursus arctos*), and to obtain short- and long-term extinction probabilities. The current study not only considers these stochastic events, but also considers harvesting rates, which can be equally important when determining the survival or extinction of an endangered species, especially when it has commercial interests (see review by Powles et al. (2000)). This is not the case in *P. ferruginea*, but as mentioned earlier, the species is commonly collected by fisherman for a wide variety of purposes.

Results were shocking in the sense that the species is expected to reach extinction in this area in 20 y if the collection rates continue at the same levels registered during 2007 to 2009, and without taking into consideration the larvae contribution to the population located in inaccessible areas in the metapopulation. The species is subject to high collection rates (as evidenced in the current study), and this prediction is congruent with the historical data available at the moment regarding the regression suffered by populations located on European coasts. Until the 19th century, the species was distributed widely in the western Mediterranean Basin, on both European and African coasts. However, since the beginning of the 20th century, in 20-y intervals, many European populations became extinct. Later, Corsica registered in 1993 an important regression of its populations in Scandola and the Cape Corse region (Laborel-Deguen et al. 1993), where almost no individuals could be found in a survey conducted in 2009 (pers. obs.). In Sardinia, where limpets are frequently recollected for various purposes (Cristo et al. 2007, Curini-Galletti pers. comm. pers. obs.), populations are also in clear regression (Porcheddu & Milella 1991), and population

densities are clearly associated with collection impact (Cristo & Caronni 2008).

Results indicate that Ceuta's metapopulation is viable if the species is not subject to collection, regardless of the high natural mortality rates registered. This could be the result, in part, of high recruitment rates. However, again, the important effect of collection rates is evidenced. Predictions indicate that if Ceuta's metapopulation was no longer subject to collection (running the simulation only with natural mortality rates), an increase in 75% of the population could be registered in 20 y. Furthermore, when simulating the effective protection of only the largest individuals (>50 mm), the metapopulation would still increase 52% during the same period of time. This could be the result of the fact that larger individuals are the population fraction most affected by collection (Rochet & Trenkel 2003, Espinosa et al. 2009a), and also contribute to a larger degree (with a greater number of oocytes) to reproduction (Levitan 1991, Tegner et al. 1996, Espinosa et al. 2006). By effectively protecting this fraction of the population, we could still guarantee the continuity of the species.

It should also be noted that these simulations were run with data obtained during a 2-y monitoring period. There are no specific studies that directly indicate how many years of monitoring are needed to infer population parameters with the precision needed for a PVA. Some authors suggest that prediction reliability increases with monitoring time, because the precision of the estimations increases with sample size (Vucetich & Waite 1998, Lotts et al. 2004). Recent studies indicate that only time series of decades produce prediction with reasonably low errors (Lotts et al. 2004). Consequently, the continuity of these monitoring studies is highly recommended. Because there are no longer time series currently available for *P. ferruginea*, the results of this study constitute a first attempt to predict the evolution of *P. ferruginea*'s metapopulation in Ceuta, which is useful for the establishment of conservation measures until more data can be collected.

Our results highlight the need to prioritize the control of harvesting rates and the obtainment of density values (along with maximum shell sizes) periodically. Moreover, the development and implementation of a specific monitoring protocol and specialized terminology are essential to the management of endangered species (Wienecke et al. 2009) such as *P. ferruginea*. This article presents the first results that will allow us to develop an adequate monitoring protocol to detect and predict possible population declines. These predictions will be useful for the implementation of specific conservation measures in time.

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