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Population Structure and Growth of the Threatened Pen Shell, *Pinna rudis* (Linnaeus, 1758) in a Western Mediterranean Marine Protected Area

E. NEBOT-COLOMER[1], M. VÁZQUEZ-LUIS[1], J. R. GARCÍA-MARCH[2] and S. DEUDERO[1]

[1] Instituto Español de Oceanografía (IEO). Centro Oceanográfico de Baleares. Muelle de Poniente s/n,
07015 Palma de Mallorca, Spain
[2] Instituto de Investigación en Medio ambiente y ciencia Marina (IMEDMAR-UCV), Universidad Católica de Valencia SVM,
C/Explanada del Puerto s/n, Calpe (Alicante), Spain

Corresponding author: maitevazquezluis@gmail.com

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Abstract

Coastal ecosystems are being extensively degraded by human activities. Benthic, slow-growing and long-lived species are highly vulnerable to these impacts. Marine protected areas may avoid biodiversity losses through habitat protection. The pen shell *Pinna rudis* is a protected species, but scarce data are available on its ecology and biology. The present study is a comprehensive ecological study encompassing several unknown aspects of the growth and inner record in relation to habitat types, density and size distribution. During the summers of 2011, 2012 and 2013, a total of 418 strip transects were conducted by scuba diving in the Marine Protected Area of Cabrera National Park (39.14° N, 2.96° E). Samples were conducted across different habitats and depths, exploring 152,146.35 m^2 in total. A large range of sizes and ages were recorded within the park with densities ranging from 0 to 6.89 ind./100 m^2. Most pen shells were patchily distributed and concentrated mainly in caves. Two hotspots represented the highest densities ever recorded worldwide, showing a potential link to high larval accumulation and settlement. The population size structure showed a unimodal distribution with shell width ranging from 6.2 to 25.0 cm, with an average shell width of 16.0 ± 3.4 cm. The absolute growth was asymptotic, with a maximum age of 28-31 years and length of 45 cm. This study on the biology and ecology of a well-established population of *Pinna rudis* in the Western Mediterranean could set a baseline for the conservation of this species in other areas.

Keywords: Bivalves, sclerochronology, age, caves, Cabrera.

Introduction

The bivalve *Pinna rudis* (Linnaeus, 1758) is distributed in patches throughout the Mediterranean Sea and Atlantic Ocean (from Santa Helena and the Gulf of Guinea to The Canary Islands, Azores, and Southern Iberian Peninsula, Poppe & Goto, 1993; Barea *et al.*, 2008). Due to anthropogenic threats, such as habitat alteration, fishing and pouching by recreational divers (Poutiers, 1987; Gómez *et al.*, 2001), this species is included in Annex II of the Bern Convention as strictly protected species and the Barcelona Convention as threatened or endangered marine species *Pinna rudis* can reach 40-50 cm in shell length (Barea *et al.*, 2008) and lives in a variety of environments such as sand, rock, coastal detritic and gravel bottoms (Poppe & Gotto, 1993) at depths ranging from 20-70m (Giacobbe & Leonardi, 1987). Although it is more commonly found among rocks and hard substrates, it is also found in *Posidonia oceanica* meadows (García-March & Kersting, 2006). Few studies about its biology and ecology exist and most of them have been focused on specific aspects, such as shell ornament (Cosentino & Giacobbe, 2006), capacity of induced shell repair (Dietl & Alexander, 2005) and the organisms associated to its shell (Wirtz & Udekem, 2001, 2008; Cosentino & Giacobbe, 2007). The knowledge gap on its biology and ecology may be linked to generally low population densities (García-March & Kersting, 2006). The species density has only been reported in the marine protected areas (MPAs) of Scandola and The Columbretes Islands (0.25 and 0.3 ind./100 m^2, respectively) (Trigos *et al.,* 2013) and in The Chafarinas Islands with a similar density to The Columbretes Islands (Guallart, 2000).

Knowledge of the population structure of a species provides useful information about its status and helps to promote conservation and management measures (Bell *et al.*, 2006). Some studies have been conducted in the MPA of Cabrera National Park to protect the native population of the Mediterranean fan mussel *Pinna nobilis* (Linnaeus 1758). These studies demonstrated that MPAs do effectively protect the species (Hendriks *et al.*, 2013; Vázquez-Luis *et al.*, 2014; Deudero *et al.*, 2015). However, none of these studies attempted to assess the spatial distribution, density and size structure of the congeneric species *P. rudis* for its conservation and management. Population structure, age and growth are poorly known for *P. rudis*. Several authors have proposed different methods to estimate growth parameters from empty shells of the congeneric species *P. nobilis* (Moreteau & Vicente, 1982; Richardson *et al.*, 1999, 2004). Recently, some authors reviewed previous methods and proposed a new method to study age and estimate growth parameters using the inner record of posterior adductor muscle scars (PAMS) (García-March & Márquez-Aliaga, 2007; García-March *et al.*, 2008; García-March *et al.*, 2011). Due to the phylogenetic proximity between *P. nobilis* and *P. rudis* (Lemer *et al.*, 2014) and the shell shape similarities, it can be assumed that the formation process of the inner record and annual posterior adductor muscle scars (PAMS) observed is similar for these congeneric bivalves (García-March *et al.,* 2011). Estimates of age and growth rates of individuals or populations have wide applications within conservation biology and in aquaculture industry (Dexter & Kowalewski, 2013). In conservation biology, knowing the age and growth rates of individuals or populations is useful to make decisions for the protection of endangered organisms. The determination of a population's growth parameters requires the establishment of a relationship between a measurable dimension of the individual and its age. The knowledge of the temporal change of that dimension permits the development of growth curves (García-March & Márquez-Aliaga, 2007). However, studying the age and growth parameters of bivalves is not straightforward, since growth can be affected by environmental conditions and varies within the species (Dexter & Kowalewski, 2013).

Given the protection status of this species, the primary interest of this study was to assess different ecological aspects, such as spatial distribution, density of individuals, size structure, age and growth in order to expand knowledge of this species in pristine habitats, such as MPAs. The aim of this study was to carry out an analysis of the population of *Pinna rudis* in Cabrera National Park. The main objectives were: i) to assess the density of individuals, distribution and size structure of the population in a MPA, ii) to estimate age and growth parameters of the population inhabiting the MPA and iii) to interpret and describe the inner record of the shells.

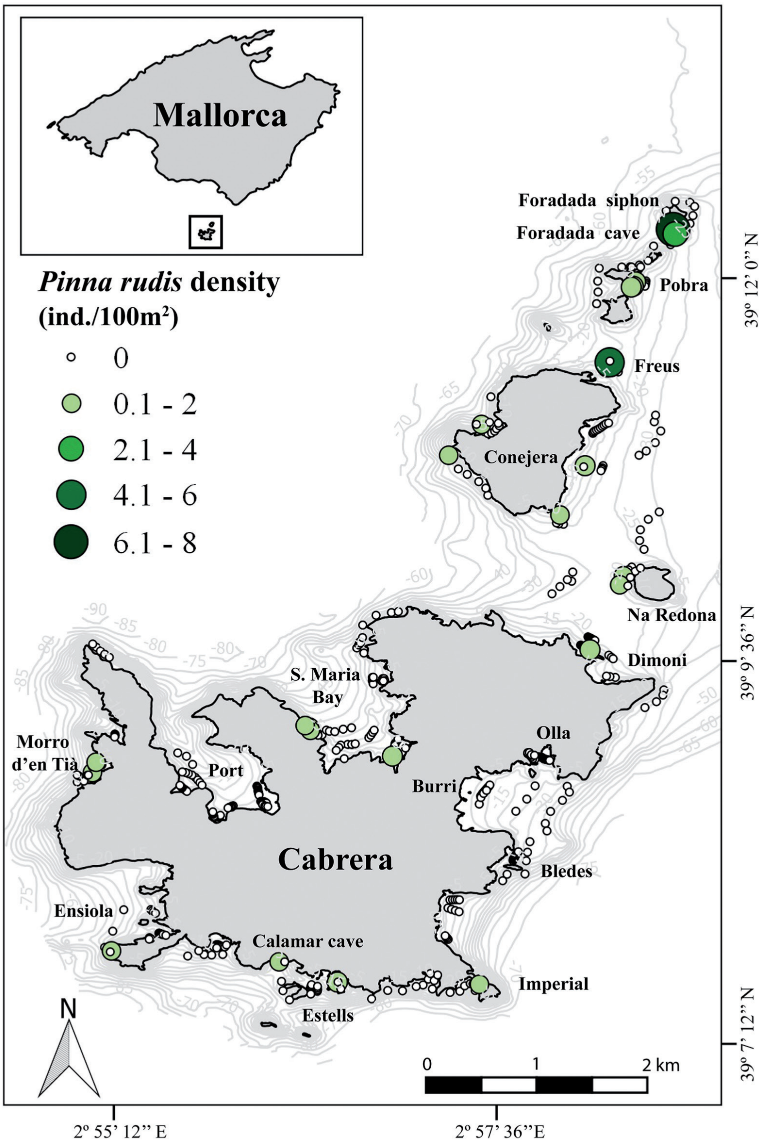

Fig. 1: Sampling locations, distribution and density of *Pinna rudis* individuals in Cabrera National Park. Points and crosses show the 418 underwater visual transects conducted in the area and bubbles indicate *Pinna rudis* densities.

Materials and Methods

Study area, data and sample collection

The study was carried out at the Archipelago of Cabrera National Park, in the south-east of Mallorca Island, Balearic Islands, Spain (39.14° N, 2.96° E, Fig. 1). The MPA was established in 1991 with a marine area of 87.03 km^2. It is comprised of 19 small islands, divided into several zones with different levels of protection. The MPA has several different habitat types such as hard rocky substrates, soft substrates, such as coastal detritic, sandy and muddy sediments, and extensive seagrass meadows of *Posidonia oceanica* located in shallow coastal waters and underwater caves found in deeper areas (20-40 m depth).

Fieldwork was carried out during the summers of 2011, 2012 and 2013. In this period, a total of 418 strip transects were conducted by scuba diving, in order to survey ecological parameters of the MPA. Data recorded were *P. rudis* density, distribution and size structure at several habitats (sand, rock, coastal detritic, caves and *P. oceanica* seagrass meadows). Depths ranged from 4 to 40 meters (Fig. 1). The length of each strip transect was 30 m and the width was 2.5 m, covering a total area of 75 m^2. 291 transects were conducted over *P. oceanica* meadows, 26 on coastal detritic bottoms, 60 on rock, 33 on sand and 8 in underwater caves (all the bottom surface of the cave). In the deepest areas (>25 m depth), strip transects were conducted with underwater scooters, and with a camera towed by a sledge to assist in surveying the area. Additionally, the staff of the Cabrera National Park was interviewed for information about the areas with higher presence of *P. rudis* based on their experience. The total surveyed surface area of all habitats was 152,146.35 m^2. 50,900 m^2 explored in *P. oceanica* meadows, 21,604 m^2 on coastal detritic bottoms, 38,415.85 m^2 on rocky bottoms, 36,866 m^2 on sandy bottoms and 4,360.5 m^2 in underwater caves. The numbers of *P. rudis* were expressed in individuals per 100 m^2 and plotted in a geographic information system (QGIS Development Team, 2009). During the exploration of the strip transects, the maximum shell width of all individuals encountered (n=113) were measured in situ using a L-shape ruler to ± 0.1 mm accuracy (Vázquez-Luis *et al*., 2014, Deudero *et al.,* 2015). Additionally, 19 empty shells from dead individuals were collected for the growth study.

Age and growth determination

To determine the age and to analyze the growth parameters of *P. rudis*, one of the valves of the empty shells collected was embedded in epoxy resin and cut dorso-ventrally in 8 cm sections to be fitted to slides. Each piece was then cut radially across the records of the PAMS (García-March *et al.*, 2011). One side of the radial sections was polished and mounted on a glass slide. A thick section (c. 200 µ) was cut using a saw (Buehler Isomet Low-Speed Saw). The free surface of the slide was polished to improve growth record observation (García-March *et al.*, 2011). From each polished section, the inner record was counted and the increments were interpreted following the methodology proposed by García-March *et al.* (2011).

Data analysis

Population structure

Differences of density distribution among habitats were assessed by applying a permutational test of a one-factor design, with habitat as fixed factor with 5 levels, caves, coastal detritic, rock, sand and seagrass. A similarity matrix was calculated for the 418 strip transects and Euclidean distance was used as similarity measure based on untransformed data. A permutational test was performed with PRIMER-E software (PRIMER; Clarke & Gorley, 2006) with the add-on package PERMANOVA+ (Anderson *et al.*, 2008).

Growth

Using the 19 dead shells collected, the growth parameters of the *P. rudis* population inhabiting the MPA were estimated. However, only the individuals that were estimated to be 3 years or older were used (n=14). Due to the higher density of living individuals in the site “Foradada” (n=9), these individuals were analyzed as a single population in order to assess if they showed different growth characteristics than those found throughout the MPA (n=14) (“All Cabrera”). The growth equation for both *P. rudis* populations were calculated through the positions of the inner record converted to total sizes (Ht). The relationship between the total size of individuals (Ht) and the length of the dorsal nacre lobe (DNL) had to be calculated (García-March *et al.*, 2011). Several authors have demonstrated a linear relationship between these two dimensions (Moreteau & Vicente, 1982; Richardson *et al.*, 1999; García-March & Márquez-Aliaga, 2007). A regression equation was calculated for all the shells collected in Cabrera National Park (n=19), including adult and juvenile individuals in order to get a more representative and reliable equation. However, due to the fact that the “Foradada” population had too low number of juveniles to create its own equation, the regression equation of the “All Cabrera” population was used in both cases. To correct for missing annual increments, especially in adult specimens, a comparison of calcite width was necessary. As García-March & Márquez-Aliaga (2007) demonstrated, the width of calcite at each annual increment is a function of the number of years deposited (i.e. age), because calcite stops growing when the nacre covers it in the inner side of the shell. Therefore, the width of calcite is a function of age. By comparing the calcite width of the three oldest records of all individuals it is possible to get an estimate of the missing records (García-March *et al*., 2011).

To estimate the growth parameters from the inner record, a Von Bertalanffy Growth Model (VBGM) was fitted to the data. This model is commonly applied in mollusk research to show a fair representation of size at age '*x*' (Bertalanffy, 1951; Pauly, 1981). It was fitted using a non-linear mixed-effects model (NLME) as proposed by Vigliola & Meekan (2009), considering that L_{max} is random and t_0 and k are fixed. This method requires knowledge of the age of the bivalves at each measurement.

All growth study calculations and analysis were carried out using R Software (R Core Team, 2013).

Results

Distribution, density and size structure of the **Pinna rudis** *population*

In general, low densities of *P. rudis* were found in the MPA. Habitat type was the main cause determining its distribution (PERMANOVA, Pseudo-F = 25.463, *P* = 0.001, Fig. 2, Table 1). A total of 88 living individuals of *P. rudis* were recorded within the 152,146.35 m^2 at depths ranging from 4.7 to 34 m, with the majority in caves (n=58). The observed average density across all habitats was 0.06 ind./100 m^2, although mean density varied greatly across the different habitats. Although caves represent only 1.91% of the total area surveyed, the mean density in caves was 1.69 ind./100 m^2. The location, Foradada Siphon, had the highest living *P. rudis* densities found in the MPA (6.89 ind./100 m^2). Between all locations, the densities varied greatly. In *P. oceanica* meadows, a mean density of 0.06 ind./100 m^2 was recorded, with densities as high as 5.33 ind./100 m^2. This habitat also had the highest density of dead individuals (4 ind./100 m^2). On rocky bottoms, coastal detritic bottoms and sandy bottoms, the mean density was 0.03 ind./100 m^2, 0.02 ind./100 m^2 and 0.01 ind./100 m^2, respectively.

Of the 113 individuals that were measured, 88 individuals were living and 25 were dead. The population size structure showed a unimodal distribution with individual's shell width ranging from 6.2 to 25 cm, with an average shell width of 15.99 ± 3.39 cm (Fig. 3). Most individuals were found in four different caves and in *P. oceanica* meadow site "Freus". In the site, "Foradada Siphon", 34 individuals were recorded, with 8.8% of these dead and an average shell width of 15.79 ± 2.01 cm. In "Imperial Cave", 15 individuals were recorded, with 20% of these dead and an average shell width of 18.15 ± 3.88 cm. In "Foradada Cave", 13 individuals were found, with 23% of these dead and an average shell width of 17.57 ± 3.51 cm. In "Freus", 40 individuals were found, with 40% of these dead and an average shell width of 13.07 ± 1.48 cm. In "Ensiola Cave 1", 11 individuals were found and a high percentage, 44%, of these dead and an average shell width of 16.98 ± 2.72 cm.

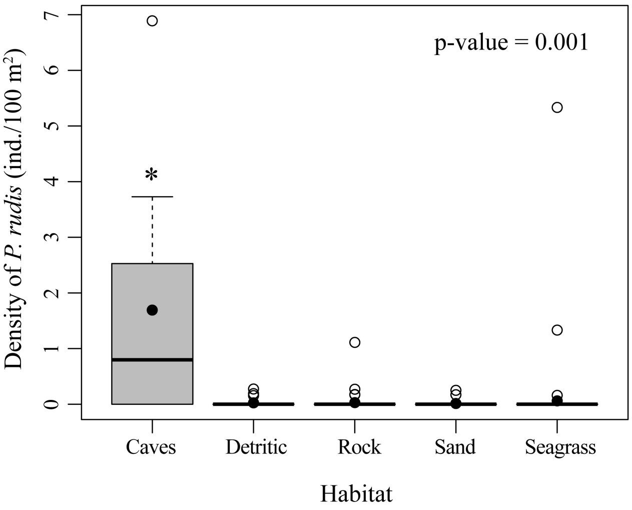

Fig. 2: Boxplot of values of *P. rudis* density (ind./100 m^2) in the studied habitats. The middle line is the median, the lower and the upper box boundaries are the first and third quartiles. The black points are the mean density of each habitat. The whiskers are the maximum and minimum observed values and the white points are the outliers. (*): C>P=R=D=Sa.

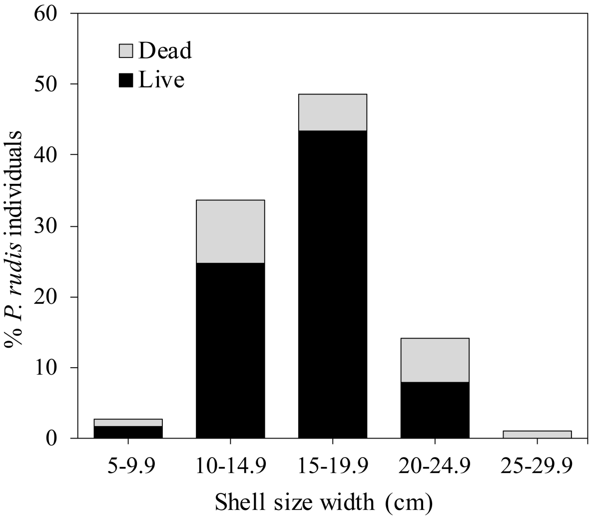

Fig. 3: Distribution of shell size structure of *P. rudis,* 88 living and 25 dead individuals.

Table 1. Results of the one-factor multivariate PERMANOVA of *P. rudis* densities between habitats. C: caves, D: detritic, P: seagrass, R: rock and Sa: sand. MS: mean square, F: F-ratio, P: p-value.

Source of variation	df	MS	Pseudo-F	P(perm)	Pair wise test
Habitat	4	5.328	25.463	0.001	C > P = R = D = Sa
Residual	413	0.209			
Total	417				

Comparison of the inner record of P. rudis *and* **Pinna nobilis**

Both congeneric bivalve species showed the same internal shell structure components described by García-March *et al*. (2011). The inner record of *Pinna rudis* is characterized by a wide miostracum layer (m), a thin nacreous layer 1 (n1) and a thin and short nacre tongue (nt), showing a clear miostracum intrusion (mi) (Fig. 4a, 4b). In contrast, the inner record of *Pinna nobilis* has a thiner miostracum, an evident miostracum intrusion, longer nacre tongues than *P. rudis* and a wider nacreous layer 1 (Fig. 4c, 4d).

Age and growth of **Pinna rudis**

Age determinations were calculated from 19 empty shells collected during the surveys. However, only 14 shells were used to calculate growth parameters, because five specimens were less than three years old. The maximum number of inner records counted in the studied shells of *P. rudis* in the National Park of Cabrera was 28 (Ensiola Cave). The six youngest individuals were estimated to be one to three years old (Table 2). The linear regression between the total size of the shell (Ht) and the length of the DNL used for both populations showed a high correlation ($Ht = 1.759 \cdot DNL + 6.362$, $R^2 = 0.966$, Fig. 5).

The growth parameters obtained with the NLME model can be observed in Table 3. Due to the fact that the t_0 values (-3.80 and -3.44) and microscopic observations of the records indicated the occurrence of several missing records in all specimens, a second growth equation was calculated for each population (yielding two equations C1 and C2 for "All Cabrera" and another two equations F1 and F2, for "Foradada"). The only difference between the two growth equations of each population occurs in the t_0 values; k and L_∞ remain unchanged. The increased number of missing records could be due to the thin nacreous layer 1 observed, which complicates the identification of the oldest records. In the second equation, individuals were estimated to be three years older (Age+3), since three years was considered as the average of missing records between individuals. This made the length at t_0 more realistic, yielding a t_0 considerably closer to 0 (Table 3).

Discussion

In this study, in order to obtain valuable information about the biology and ecology of the threatened species *P. rudis* a large sampling effort throughout different habitats and depths along the MPA of Archipelago of Cabrera National Park was carried out. The data will help to set the basis for the establishment of specific future conservation measures. Results from this study are comparable with previous studies carried out in other MPAs (0.25 ind./100 m^2, in Scandola and 0.30 ind./100 m^2 in Columbretes; Trigos *et al*., 2013). Densities of *P. rudis* within all habitats were low. However, in this study the area surveyed was larger than previous studies. *P. rudis* showed preference for caves and hard substrate habitats, since most of the individuals were found in detritic bottoms of caves.

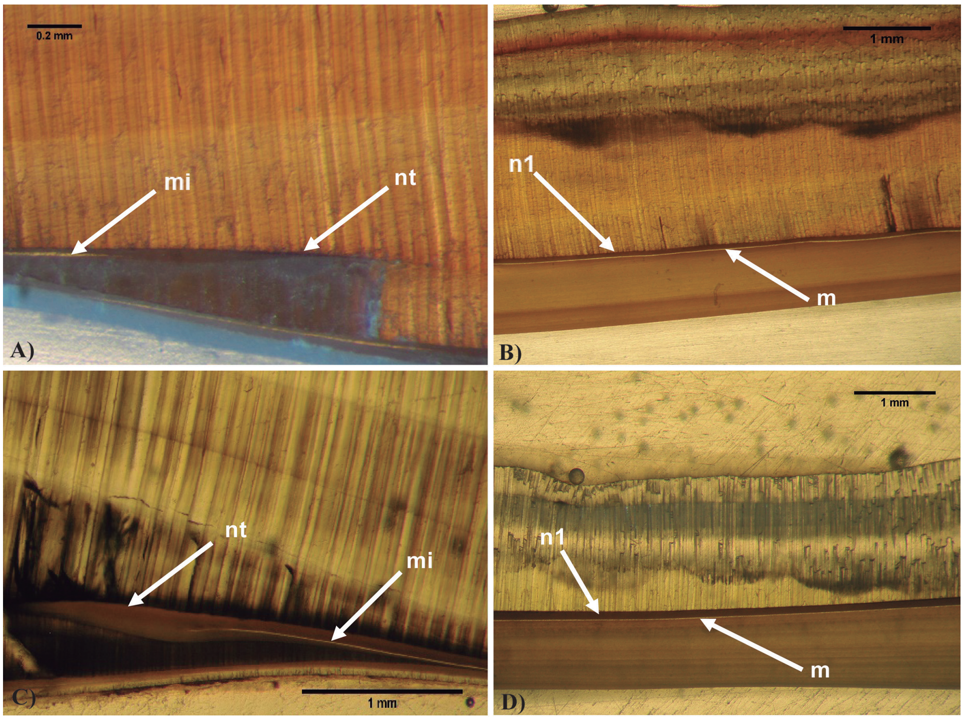

Fig. 4: **A, B** Inner record of *Pinna rudis* shells, **C, D.** inner record of *Pinna nobilis*. Abreviations: nt, nacre tongue; m, miostracum layer; mi, miostracum intrusion; n1, nacreous layer 1.

Table 2. Summary of locality, age, lengths and widths of the 19 shells of *Pinna rudis* collected from Cabrera National Park. Habitat: C: cave, P: *Posidonia oceanica* meadows, R: rock. [a]: shells collected but not included in the population growth equation, [b]: this *P. oceanica* meadow was growing over a rocky platform. Age: real number of nacre tongues counted, Age +3: real number of nacre tongues counted plus 3 years.

Locality	ID	Mean depth (m)	Habitat	Length (cm)	Width (cm)	Age	Age +3
Ensiola Cave	Ensiola1[a]	30	C	18.8	12.3	2	5
	Ensiola2	30	C	42.7	22.25	28	31
	Ensiola3	30	C	42.6	18.6	13	16
Cala Gandulf	Gandulf 1	10	P	21	10.3	3	6
	Gandulf 2[a]	10	P	11.3	5.7	1	4
Imperial Cave	Imperial1	25	C	40.7	22.45	18	21
	Imperial2[a]	25	C	19.9	13.2	2	5
	Imperial3[a]	25	C	18.15	9.2	2	5
Foradada Siphon	Foradada Siphon1	30.4	C	43	17.9	18	21
	Foradada Siphon2	30.4	C	38.9	18.5	12	15
	Foradada Siphon3	30.4	C	40.2	19.5	13	16
	Foradada Siphon4	30.4	C	44.2	20.7	21	24
	Foradada Siphon5	30.4	C	34.7	17.3	8	11
	Foradada Siphon6	30.4	C	39.8	21.7	23	26
	Foradada Siphon7	30.4	C	37.9	20.7	19	22
Foradada Cave	Foradada Cave1	26.2	C	42.3	18.9	9	12
	Foradada Cave2	26.2	C	38.3	20.7	25	28
Freus	Freus[b]	10	P	26.1	10.8	7	10
Conejera	Conejera[a]	10	R	18.8	8.3	2	5

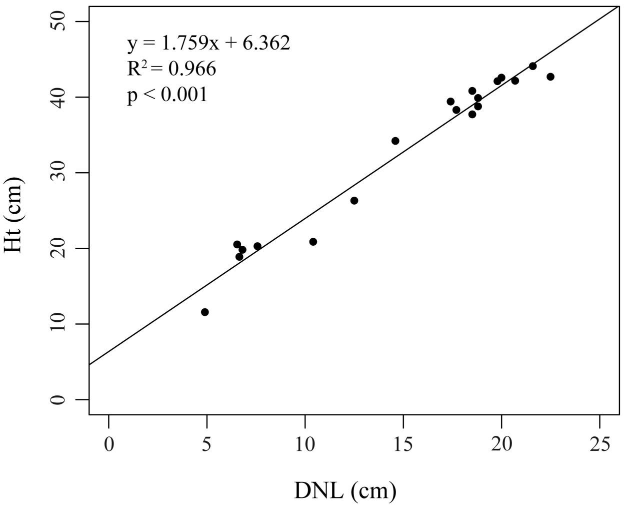

Fig. 5: Relationship between the length of the shell (Ht) and the length of the dorsal nacre lobe (DNL).

The explanation of such distribution may be related with the microstructure of the shell. The inner record of *P. rudis* has a thin nacreous layer (this layer is much thinner in *P. rudis* than in *P. nobilis*). According to Carter (1990), the nacreous layer provides resistance to shell breakage in Pinnids. Detritic bottoms and caves could give more stability and support for settlement and growth of *P. rudis* than shallower open habitats. Observations in the Strait of Messina showed similar patterns, where *P. rudis* was found living in deeper areas (20-70 m) over detritic bottoms under the influence of the deep currents (Giacobbe & Leonardi, 1987). Caves exhibited the highest density of *P. rudis*, although this habitat represents only 1.91 % of the total area explored. Highest densities were found in “Foradada Siphon” (6.89 ind./100 m^2), a tubular funnel-shaped cave, with coastal detritic bottoms ranging from 26 to 34.5 m depth. This is the highest density recorded worldwide, indicating that this is an optimum area for

Table 3. Results of growth parameters for both populations (C: All Cabrera, F: Foradada). k: speed at which the maximum asymptotic size of the population is reached, L_{max}: maximum length of the population, n: number of individuals within the population, t_0: age at length 0.

Equation	Populations	k	t_0	L_{max}	n
C1	All Cabrera	0.14	-3.80	45.27	14
C2	All Cabrera +3	0.14	-0.80	45.27	14
F1	Foradada	0.15	-3.44	45.94	9
F2	Foradada +3	0.15	-0.44	45.94	9

preferential accumulation and settlement of larvae. The density of individuals in *Posidonia oceanica* meadows was variable ranging from 0 ind./100 m^2 to 5.3 ind./100 m^2. The effect of strong bottom currents may explain high densities, such as those found in "Freus", because of the beneficial nutrient renewal and optimal conditions for the settlement and growth of new individuals. However, these conditions could also increase mortality due to fatigue, explaining the high amount of dead individuals in "Freus" (4 ind./100 m^2). Similar processes have been reported for the congeneric species *P. nobilis* in other shallow areas (García-March *et al.*, 2007). On the other hand, some differences in average shell width were detected among locations. The average shell width in "Freus" was 3 cm lower than all other populations. As Zavodnik (1967), Vicente *et al.* (1980) and García-March et al., (2007) noticed, the size distribution of *P. nobilis* is related to water depth, where small specimens are more abundant in shallow areas and the larger ones are predominate in deep sites. Similar effects probably occur in *P. rudis* populations. In order to better understand the causes of the peculiar population structure of this species, it is advisable to incorporate additional oceanographic data in future studies.

Regarding the age and growth of *P. rudis*, a well-established population inhabits the MPA with a large range of individuals of different sizes and ages. The majority of individuals are mature adults, 10-20 years old. These breeders are essential for the spawning and recruitment of this species (Coppa *et al.*, 2010). Growth parameters of the pen shell did not differ spatially. The maximum age range of *P. rudis* found in "All Cabrera" surveyed stations was 28-31 years and in "Foradada", 25-28 years. Additionally, both groups showed similar and high asymptotic growth (L_∞ = 45-46 cm), and presented a moderate rate at which the asymptotic maximum size was reached (*k* = 0.14-0.15). A similar growth study on *P. nobilis* was carried out in two specific areas of Cabrera, showing differences of age, growth speed and maximum shell size between the exposed "Freus" and sheltered "Gandulf" areas (Martínez *et al.*, 2014). Despite, the small number of *P. rudis* shells collected in each area preventing a proper assessment of the effects of exposure, it was possible to find differences in growth speed and maximum size between both species. *P. nobilis* showed faster growth (*k* = 0.19-0.21) and larger maximum size (37.1-64.6 cm) (Martínez *et al*., 2014) than *P. rudis*. These differences on growth parameters between species are probably related to the biology and ecology of each species. To further explore this, future studies with larger sample sizes in different areas should be carried out.

Concerning the inner record of the shell, several PAMS are missing in the anterior oldest part of the shell, especially in adult specimens of *P. nobilis* (García-March *et al.*, 2011). This pattern is similar in *P. rudis*. Despite both species inner records sharing all components described by García-March *et al.* (2011), in *P. rudis* the nacreous layer 1 and the nacre tongue are thinner than in *P. nobilis*. This adds some uncertainty in the determination of PAMS, as some of the oldest PAMS are nearly unrecognizable in *P. rudis*. The oldest PAMS were discarded when their position was unsure, despite data indicated that some PAMS should still be present. There is a possibility that at least three PAMS were missing and an average between each specimen and considering the t_0 value was taken into consideration. To account for this potential bias, two equations were calculated for each population. In the first equation (C1 and F1) growth parameters were calculated using the number of nacre tongues counted adding the missing records estimated using calcite width. In the second equation (C2 and F2), the growth parameters were calculated assuming that all individuals were three years older after estimating missing records using calcite width. It should be noted that the process of estimation of missing records using calcite width is unable to predict records that are unreadable in all specimens. Due to this, the oldest observable record of all specimens is the starting point for the cross dating among individuals. In the first equation, the t_0 value was strongly negative for both groups (-3.80 and -3.44 years). This implies an unrealistic initial size of *P. rudis* at the time 0 to be 17.70 to 18.80 cm. In the second equation, the value of t_0 decreases very close to 0 and the initial size of *P. rudis* at time 0 is around 2.80 and 4.80 cm for both groups, this is a closer value to in situ observations. This suggests that due to the thin nacreous layer 1 of *P. rudis*, the oldest records that corresponded to records when the animal was younger are not visible with binocular lens. Thus, the age and growth parameters calculated with the second equation fit more rigorously with the growth of *P. rudis* in the Cabrera National Park. In any case, given the shell structure differences between *P. rudis* and *P. nobilis*, the results obtained regarding the addition of three years should be interpreted with caution. One way to confirm the existence of missing records in the anterior part would be analyzing oxygen stable isotopes in the oldest parts of the shell. The observation of seasonal peaks and valleys in the time series would demonstrate the presence of the records. Additionally, Carter (1990) confirmed that nacre in columns allows higher resistance to breakage due to impact. Therefore, the differences in the width of the nacreous layer between both species could make *P. rudis* more sensitive to shell breakage and consequently make the PAMS identification more difficult in the anterior part of the shell.

In conclusion, this study provides quantitative information on the population structure, age and growth of the pen shell in a marine protected area. This valuable information provides the first step for the continued preservation and maintenance of this stable, healthy and almost unthreatened population of pen shells. Due to the low densities observed, each individual is important for the sustainability of the population. Thus, it should be a priority to establish a monitoring program within the MPA, specifically in the areas of highest density ("Siphon" and "Freus") to obtain long-term demographic data. Additionally, the implementation of an integral reserve and no-take area in the highest density sites is highly recommended for conservation and management purposes.

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