



Habitat use by a large population of *Pinna nobilis* in shallow waters

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Summary: We investigated the habitat use and size structure of the fan mussel, *Pinna nobilis* L. in the Alfacs Bay (Ebro Delta, Spain, NW Mediterranean). Shore-parallel transects were conducted to assess the abundance, size, and orientation of individuals and to record habitat features along the Banya Sandspit, at depths of 20 to 130 cm. Results showed two distinctive areas in terms of population density, marked by the end of local salt pan dikes that we named Good Habitat (GH) and Bad Habitat (BH). To extrapolate these results to the full area of BH and GH, perpendicular transects were conducted to determine the local bathymetry. Then, the underestimation of individuals due to the effects of distance, depth and seagrass cover (Distance 6 computer package) was added to raw abundances to obtain a corrected population of 90303 individuals (12085 in the BH and 782018 in the GH), the largest so far reported at such low depths. Most recorded individuals were adults (40 to 60 cm shell length), with no occurrence of sizes <20 cm, and with a strong association with *Cymodocea nodosa* seagrass beds. Given the shallow distribution of the population, the absence of small sizes during the study period, and the presence of human activities that may damage *P. nobilis* and its habitat, the development of a management plan appears imperative for the conservation of the species.

Keywords: fan mussel; endangered species; *Cymodocea nodosa*; habitat use; water depth; human activities.

Uso del hábitat por una gran población superficial de *Pinna nobilis*

Resumen: El uso del hábitat y la estructura poblacional de la nacra, *Pinna nobilis* L. fue investigado en la bahía dels Alfacs (Delta del Ebro, Spain, Mediterráneo noroccidental). La abundancia, talla, y orientación de los individuos, así como las características del hábitat se evaluaron mediante transectos paralelos a la costa, a profundidades comprendidas entre los 20 y los 130 cm. Los resultados evidenciaron la presencia de dos áreas con densidades poblacionales diferenciadas, delimitadas por el final de los diques de las salinas locales, a las que se denominó Good Habitat (GH) y Bad Habitat (BH). Para extrapolar los resultados a toda el área del BH y el GH, se realizaron transectos perpendiculares para determinar la batimetría local. A continuación, las tasas de infravaloración de individuos debido al efecto de la distancia, la profundidad, y la cobertura de fanerógamas marinas obtenidas con el paquete informático Distance 6 se añadieron a los valores no corregidos, y resultaron en una estima de 90.303 individuos (12.085 en el BH y 78.2018 en el GH, la mayor hasta ahora registrada en aguas poco profundas). La mayoría de los individuos observados eran adultos (40 a 60 cm de longitud de valvas), sin ocurrencia de tallas <20 cm, y con una fuerte asociación a la pradera de *Cymodocea nodosa*. Dada su distribución poco profunda, la ausencia de individuos juveniles durante el periodo de estudio, y la presencia de actividades humanas en la zona que potencialmente pueden perjudicar a *P. nobilis* y a su hábitat, la implantación de un plan de gestión es prioritario para la conservación de la población local.

Palabras clave: nacra; especies amenazadas; *Cymodocea nodosa*; uso del hábitat; profundidad; actividad humana.

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INTRODUCTION

The fan mussel, *Pinna nobilis* L., is among the largest bivalves in the world, and the largest in the Mediterranean, attaining lengths of up to 120 cm (Zavodnik et

al. 1991) and reaching an age of more than 20 years (Butler et al. 1993, Galinou-Mitsoudi et al. 2006). The species suffered a serious decline about 20 to 30 years ago, mostly as a result of human activities such as persistent trawling and anchoring on nearby shore seagrass

habitats and collection of shells for ornamental purposes (Richardson et al. 1999). As a consequence, *P. nobilis* was listed as endangered in the Mediterranean Sea (Barcelona Convention, protocol ASPIM Annex 2) and is also strictly protected from all forms of deliberate capture or killing by the European Council Habitats Directive 92/43/EEC on the Conservation of Natural Habitats and the Wild Fauna and Flora (Habitats Directive Annex IV of EEC 1992). At present, despite decades of combined legal species and habitat protection, common abundances of *P. nobilis* are less than 1 individual per 100 m⁻² (Guallart and Templado 2012), although aggregations of more than 20 individuals per 100 m⁻² have been observed in some areas where the species is still abundant (e.g. Siletic and Peharda 2003, Rabaoui et al. 2008, 2010, Addis et al. 2009).

The species distribution typically follows that of seagrass meadows of *Posidonia oceanica*, but to a lesser extent it also occurs on other soft-bottom habitats such as *Cymodocea nodosa* meadows and macroalgal beds and/or sandy and muddy shores in estuaries (Katsanevakis 2006, Guallart and Templado 2010, 2012). Individuals of *Pinna nobilis* can be found throughout an extensive depth range of 0.5 and 60 m (Butler et al. 1993), although population maximums are usually reported from ca. 3–4 m to ca. 14–15 m (García-March and Kersting 2006, Katsanevakis 2007, Coppa et al. 2013). To our knowledge, populations peaking at depths of less than 1 m are rare and have only been reported once in a Tunisian coastal lagoon (see Zakhama-Sraieb et al. 2011). The spatial distribution also seems to be subjected to depth-size segregation, with very few large individuals in shallow and exposed areas, higher numbers at increasing depths (Addis et al. 2009, Guallart and Templado 2012), and higher numbers of juveniles in shallow areas (Katsanevakis 2006). Although the mechanism(s) for size segregation is not well-understood, it has been hypothesized to occur as a result of the movement of young individuals capable of releasing and regenerating byssus threads or as a result of depth-selective predation or hydrodynamic stress (García-March 2005). In addition, shallow depths may induce the development of “dwarf” adults, thus preventing size-age comparisons among individuals of the same population inhabiting different depths (García-March et al. 2007a).

The investigation of large underwater areas is often complicated by the large depth distribution of *P. nobilis* populations; individuals need to be carefully located by scuba diving and survey performance becomes constricted by working time, which involves great sampling efforts (Richardson et al. 2004). Possibly for this reason, most studies indicating high *P. nobilis* densities have only been carried out in areas smaller than 10000 m⁻² and the total population reported is usually much lower than 1000 individuals (e.g. Galinou-Mitsoudi et al. 2006, García-March and Kersting 2006, García-March et al. 2007a, Siletic and Peharda 2003, Addis et al. 2009, Zakhama-Sraieb et al. 2011). More exceptionally, some studies have explored areas larger than 100000 m⁻² and reported populations of thousands of individuals, in addition to very high

local densities in discrete areas (Katsanevakis 2006, Katsanevakis and Thessalou-Legaki 2009). Nevertheless, very small populations are in extreme jeopardy of falling prey to demographic stochasticity, studies at larger spatial scales are therefore necessary to assess the status of this endangered species and prevent the loss of genetic diversity.

The Alfacs Bay is a large, semi-enclosed estuarine water body located at the south of the Ebro River delta, NW Mediterranean. The bay is very important for the economy of the region due to its fish and shellfish aquaculture, particularly of oysters and mussels (Solé et al. 2009), and it receives seasonal freshwater inputs (ca. 275 10⁶ m³ yr⁻¹) from rice cultivation between April and October (Garcés et al. 1999). The shallow submerged platform along the inner shore (<2 m) is dominated by patches of the seagrass *C. nodosa* that are recurrently disturbed by the migration of subaqueous dunes (Marbà and Duarte 1995) and follow a steady-state equilibrium between extinction and recolonization (Vidondo et al. 1997). The southern shore (the Banya Sandspit) was included in the Ebro Delta Natural Park in 1986 and is also part of the Natura 2000 network of the European Union because of its importance for both *C. nodosa* and *P. nobilis* (Ibáñez 1997), according to its status in the Red Book of Endangered Species for the Spanish waters (Guallart and Templado 2012). Despite this protection status, approximately half of the Banya Sandspit is occupied by a salt factory with extensive salt pans, and recreational and fishing boats are free to access the area and anchor along the entire shoreline. In addition, there is also a ferry operating regularly between the port in Sant Carles de la Ràpita and a snack bar located close to the mouth of the bay that attracts high numbers of tourists to the area during the summer period.

Although *P. nobilis* is mentioned in the non-scientific literature and is well-known by local fishermen, this study provides the first scientific record of the species and its habitat uses in the Ebro Delta. According to Guallart and Templado (2012), the nearest populations of *P. nobilis* occur at the locality of L'Ametlla de Mar (ca. 35 km north of the Ebro Delta) and in Peñíscola (ca. 40 km south of the Ebro Delta). The objectives of this study were: (1) to estimate the population size, density, size structure, and distribution of *P. nobilis* in the Alfacs Bay; (2) to assess the influence of habitat features on the abundance of individuals; and (3) to assess the potential negative effects of human activities on the population, including quantification of direct damage to individuals by boating activities, and the presence of distribution patterns of that could be attributed to agricultural eutrophication and salt extraction.

MATERIALS AND METHODS

Study area

The Alfacs Bay is a semi-confined estuarine area of ca. 49 km² with an average depth of 3.13 m located on the southern part of the Ebro Delta (40°36'39" N, 0°39'25" E; Catalonia, NW Mediterranean). It is

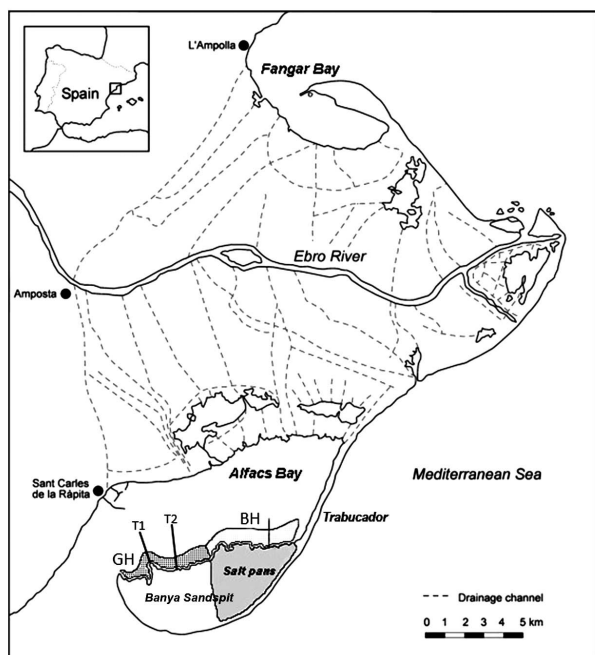


Fig. 1. – Map of the Ebro Delta, showing the position of the Banyas Sandspit on the southern shore of the Alfacs Bay, and the area of distribution of *Pinna nobilis*. Areas of BH and GH, and the two across-shore transects along the depth gradient (0 to ca. 6 m) are indicated.

bordered by rice fields to the north and connected to the Banyas Sandspit through a long sand bar known as the Trabucador that stretches along the eastern side of the bay (see Fig. 1). The mouth of the bay is ca. 2.5 km wide and its edges are surrounded by a shallow platform of 18 km² (ca. 700 m wide), which gently slopes from 0 to 1.5 m, with an average depth of 0.64 m (Garcés et al. 1999). Away from the edge, the platform leads to a more pronounced slope that descends to the muddy central basin at a maximum depth of 6.5 m. The northern part of the platform occupies 6 km² and is composed of silty sediments, whereas the eastern and southern sides, which occupy over 12 km², are sandy (Garcés et al. 1999). The northern edge of the bay is connected to irrigation channels from rice fields (Fig. 1) that bring significant amounts of nutrients and organic matter that favour the development of phytoplankton (Delgado 1987) and fast growing macroalgal communities (*Chaetomorpha linum* and *Ulva* spp. P. Prado, pers. observ.) and decrease salinity in shallow waters (0 to 2-3 m depth) to ranges between 30‰ and 35‰ (Garcés et al. 1999). In contrast, the salinity at the southern edge of the bay is more similar to the open sea and macroalgae are mostly constricted to sparse patches of *Caulerpa prolifera*.

Field surveys

We conducted a preliminary visual survey from a boat with three observers to determine which areas of the Alfacs Bay hosted *Pinna nobilis*. Some surveys were also conducted in the northern Bay of El Fangar (Fig. 1), but no individuals were observed. On the northern shore of the bay, only a few isolated individu-

als were observed at the eastern end, with none found along the Trabucador sand bar. In contrast, increasing numbers of individuals were detected along the Banyas Sandspit (southern shore of the bay), particularly from the north end of the salt pan area to the mouth of the bay. After the visual surveys, we conducted two across-shore transects along the depth gradient (0 to ca. 6 m) using scuba to define the depth range where individuals could be detected (Fig. 1). The population of *P. nobilis* was then studied in a total of 102 shore-parallel transects along the Banyas Sandspit in the summer of 2011-2012. This survey covered a total length of ca. 10.5 km, at depths ranging from 0.1 to 1.3 m and covering the full depth range of the shallow shelf. After data analyses, the area containing *P. nobilis* was divided into two regions with distinctive abundance of individuals and delimited by the end of the salt pans that we named *Bad Habitat* (BH; from the Trabucador sandbar to the end of the salt pans) and *Good Habitat* (GH; from the end of the salt pans to the mouth of the bay). Line transects (ca. 100 m each) were established consecutively from the end of the former transect, starting on the shore of the Trabucador, and coordinates were saved on a GPS receiver. Individuals were counted by two wading observers, although sizes <10 cm (i.e. individuals belonging to the first size class; Katsanevakis 2006), might have not been detected by eyesight or direct encounter. A third observer on board a support boat using a YSI 6660 V2 probe equipped with a 650 MDS data logger was responsible for data logging and recording of depth, seagrass cover, oxygen levels, temperature and salinity at the beginning and at the end of each transect. Differences in environmental variables between the two habitats were investigated using a Student's t-test for independent samples.

In addition, for each observed individual, the following variables were recorded: the perpendicular distance from the transect line, shell width and height from the sediment surface, valve orientation, depth, sediment type and seagrass cover (semi-quantitative estimation). Shell width was measured in situ with aluminum vernier calipers, and for depth and perpendicular distance from the line we used a metre stick and a 10-m tape measure, respectively. The geographical orientation (0-180°) of the line of valve opening of each live specimen was measured with an underwater compass. The number of dead individuals and/or those showing proppeller scars per transect were also recorded.

Dead individuals found intact were measured for shell width (*W*) and full length (*L*) from the umbo to the ventral side, and the data were used to build a function for estimating the total length of living individuals buried on the seafloor and to determine the size distribution of the population. The allometric growth of *W* in relation to *L* was investigated using the $\ln W = f(\ln L)$ relationship for a quadratic model indicated for *P. nobilis* in Rabaoui et al. (2007). Then, the Levenberg-Marquardt algorithm was used as a method for least-squares estimation of non-linear parameters. All measurements were conducted during a 3-month period, in August-September 2011 and in June 2012.

Estimation of habitat size by depth intervals

To extrapolate our results from shore-parallel transects to the population within the whole Banyas Sandspit, the bathymetry within the suitable habitat of *P. nobilis* was assessed by conducting 10 transects perpendicular to the southern shore of the bay and ca. 1 km apart. Transects were conducted from 130 cm depth to the shoreline with measures taken every 50 m up to a total of 230 georeferenced points. Then, the area of *P. nobilis* distribution in the GH and BH regions was estimated within the area between 20 and 130 cm depth using the area tool available in Google Earth Pro (Google, Mountain View, CA, USA). Then, the area of GH and BH by depth intervals of 10 cm was estimated as the percentage of points at each depth times the total estimated surface of each habitat.

Population estimates

The area of shore-parallel transects per 10-cm interval was estimated as the frequency of points at each depth range in the GH and in the BH (transect data values; 50 in the BH and 51 in the GH) times the total transect area sampled within each habitat (i.e. length of all transects in each habitat \times twice the transect half-width given by Distance 6; see below). Then, the abundance of individuals per m² by depth interval was estimated as the quotient between the number of individuals found at each depth (50 in the BH and 1018 in the GH) and the estimated area at each interval. Finally, densities were multiplied by the area of GH or BH at each depth interval to obtain abundance values, uncorrected for the effect of distance and environmental factors affecting visual census.

The Distance 6 computer package (Laake et al. 1994) was used to assess the possible underestimation of observer's transect counts induced by distance and by environmental factors (covariates) such as seagrass cover and depth. This multiple covariate distance sampling (MCDS) approach was conducted separately for each habitat region (BH and GH) in order to obtain more accurate deviations of population estimates. Depth was selected as a covariate because it affects water transparency, and the presence of the seagrass *C. nodosa* was considered to account for the possibility of *P. nobilis* hiding under the leaf canopy. The size of individuals, however, was not included as covariate since observed individuals were very homogeneous in size.

The MCDS approach uses the distribution of the observed distances from the transect line to individuals and data of the covariates to estimate the probability of detecting *P. nobilis* as a function of distance. The different key functions suggested in MCDS by the Distance software are the half-normal and the hazard rate with cosine, simple polynomial and Hermite polynomial; possible combinations of series expansion terms or adjustment terms were all run in order to obtain the most robust estimates of density (Buckland et al. 2001). Selection of the appropriate key function and series expansion for a given data set ultimately relies on model selection criteria such as the small-sample,

bias-corrected form AICc (Hurvich and Tsai 1989) of Akaike's Information Criterion (AIC) (Akaike 1985). The model with the smallest AICc value (AICc,min) was selected as the best among the models tested. The AICc differences, $\Delta_i = AICc_i - AICc_{min}$, were computed for all candidate models. According to Burnham and Anderson (2002), models with $\Delta_i > 10$ have essentially no support and can be omitted from further consideration, models with $\Delta_i < 2$ have substantial support, and models with $4 < \Delta_i < 7$ have considerably less support. In addition, the program produces goodness of fit tests (Kolmogorov-Smirnov and chi-square) to evaluate the fit of the model. Right-truncation was explored as a method for removing outlying records at further distances (Buckland et al. 2001) but did not improve estimates of population density or accuracy, so the largest distance observed from the transect line was maintained as the transect half-width.

Among output variables given by Distance 6, the following were included: transect half-width (used in previous calculations), density of individuals per m², and the total number of individuals within the area of specification (line transects). The latter was used to compare with the number of individuals counted by the two observers and thus to assess the underestimation resulting from distance and covariates. Then, this underestimation was added to uncorrected measures of abundance in each habitat region and depth interval to obtain a final estimate of the population size.

RESULTS

Habitat features

The two preliminary across-shore transects found that individuals were distributed between depths of ca. 20 and 130 cm water depth, with peaks at 70-80 cm (Transect 2) and 90-100 cm (Transect 1) and no significant differences in the observed number of individuals between transects ($t=1.81$; $df=10$; $p>0.05$; see Fig. 2). In addition, the curve for habitat use through

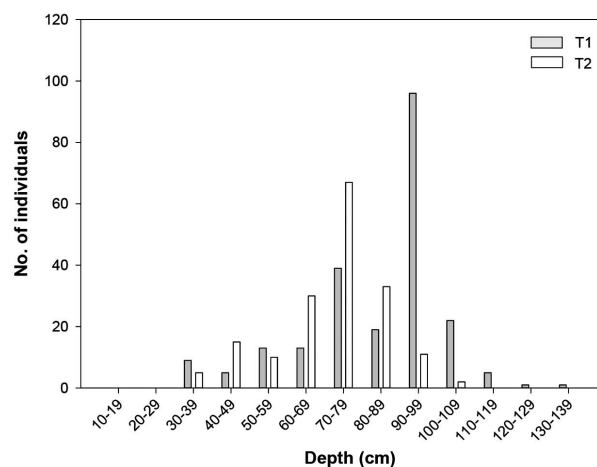


Fig. 2. – Number of individuals observed in the two across-shore transects conducted from 0 to 6 m (max. depth of the bay). No individuals were found shallower than 29 cm or deeper than 139 cm depth.

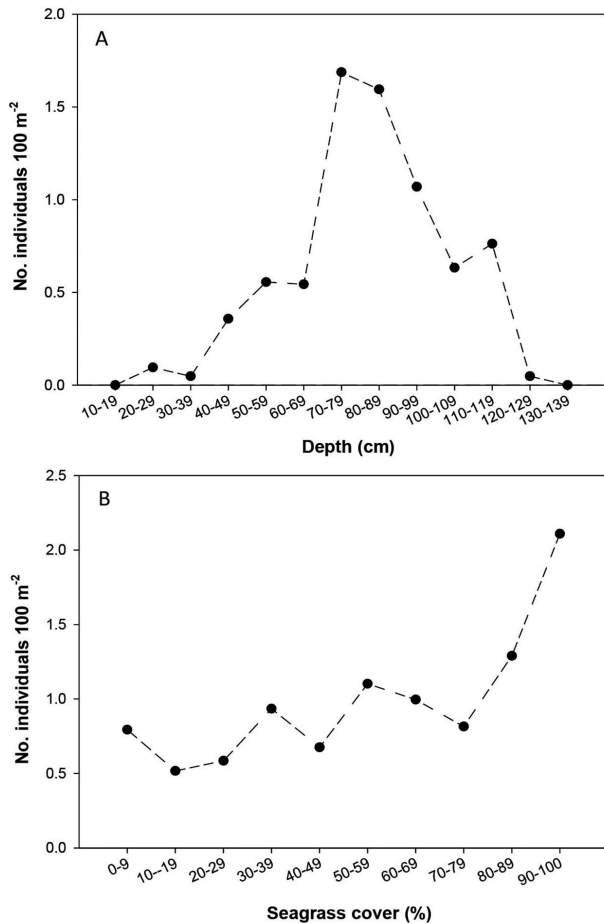


Fig. 3. – Effect of habitat features on the abundance of individuals (ind./100 m²) within line transects; A, depth; B, seagrass cover.

depth ranges across the entire Sandspit (i.e. GH and BH) showed a clear density peak at 70 to 90 cm (1.59 to 1.69 ind./100 m²). For the seagrass *Cymodocea nodosa*, no evident effect on the abundance of individuals was observed until cover reached values of 80%-90%, and it caused an increase of ca. 40% at maximum values of 90%-100% (ca. 2.1 ind./100 m²) (see Fig. 3).

Significant differences in depth between BH and GH regions were found (85.3±3.1 and 73.3±2.6 cm, in the BH and the GH, respectively) (t=2.92; df=98; p<0.001). In contrast, seagrass cover (46.2±4.3 and 48.7±4.1% in the GH and the BH, respectively), and salinity (35.9±0.15 and 36.3±0.05 in the GH and the BH, respectively) showed no significant differences (t=1.6; df=98; p>0.05 for both cover and salinity). Temperature and oxygen levels showed considerable temporal variation but mean values varied between 27 and 24°C and 92% and 100% in the BH and the GH, respectively.

Estimates of density and population size

The frequency of detected individuals decreased with increasing distance from the observers up to a transect width of 2×2 m in the BH and 2×7.9 m in the GH (Fig. 4). The 50 transects conducted along the BH covered an approximate distance of 5263.2 m along the

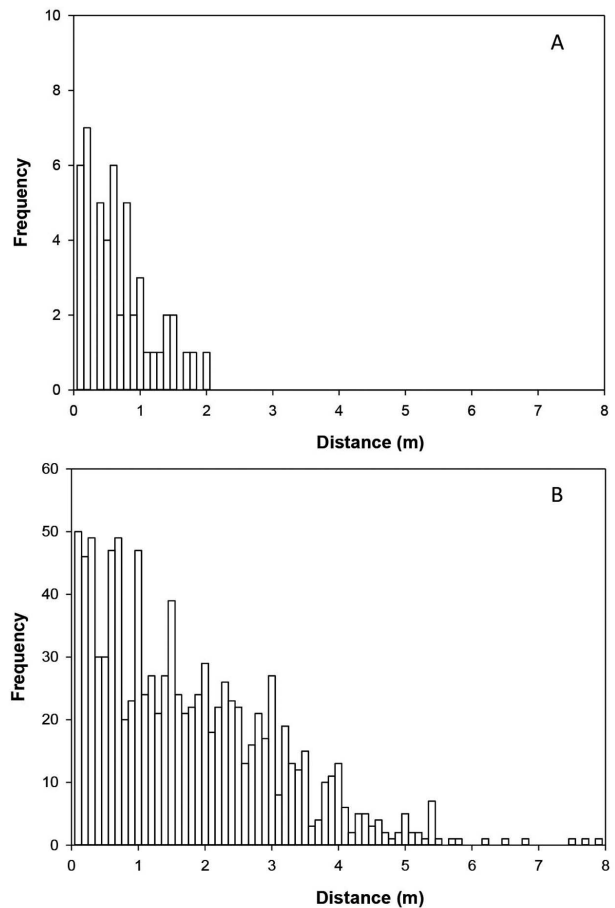


Fig. 4. – Frequency histogram of distance values from the transect line at which individuals were detected during shore-parallel transects in the BH (A) and in the GH (B).

coast, with only 50 individuals of *P. nobilis* observed that just appeared at depths of 67 to 117 cm (Fig. 5). In the GH, the 51 transects conducted covered a similar distance along the coast (5382.17 m) but 1018 individuals were observed across depth ranges of 28 to 130 cm (Fig. 5). In addition, we found 22 dead individuals, 3 in the BH and 19 in the GH (ca. 2% of the total observed), as well as 11 individuals that had been pulled up out of the sediments (ca. 1% of the total), and up to 54 that had been scarred by boat propellers (ca. 5% of the total).

The area of the BH sampled from the 10 perpendicular transects along the Banya Sandspit was larger than that of the GH (4153100 and 2943200 m², respectively). Extrapolation of line transect abundances per depth range to the available area of BH and GH at each depth produced a raw estimate of 31683 individuals (5678 individuals in the BH and 26005 individuals in the GH), to which Distance 6 model corrections were added (see next).

Model selection for evaluation of the effects of distance, depth and seagrass cover on population estimates based on the difference between the AICc of tested models and the smallest AICc provided values of 2.67 and 0.021 for the BH and the GH, respectively, and were considered to have substantial support. In both habitats, the best model for the detection function

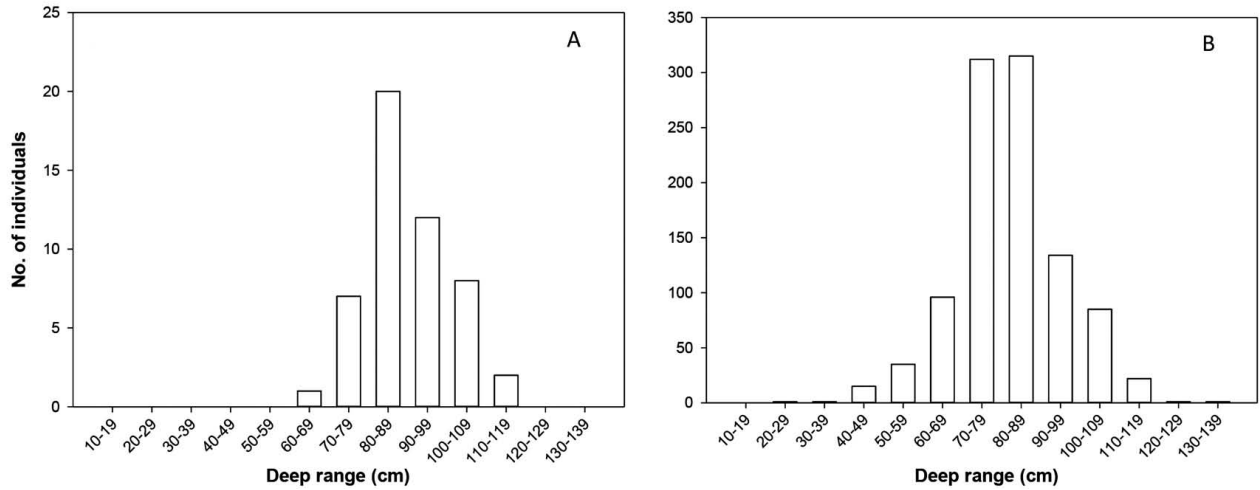


Fig. 5. – Number of individuals observed by depth intervals during shore-parallel transects in the BH (A) and in the GH (B).

of *P. nobilis* individuals given by Distance 6 was the half-normal function with no series expansion:

$$g_s(y) = \exp(-0.520 y^2) \text{ in the BH}$$

$$g_s(y) = \exp(-0.106 y^2) \text{ in the GH}$$

Neither Kolmogorov-Smirnov (BH: $D_n=0.1036$; $p=0.6571$, GH: $D_n=0.0266$; $p=0.4670$) nor chi-square tests (BH: $\chi^2=4.21$; $df=4$; $p=0.377$, GH: $\chi^2=37.7$; $df=23$; $p=0.057$) evidenced any significant deviation in the fit of the model. In the two habitats, the resulting detection function proved to be strongly influenced

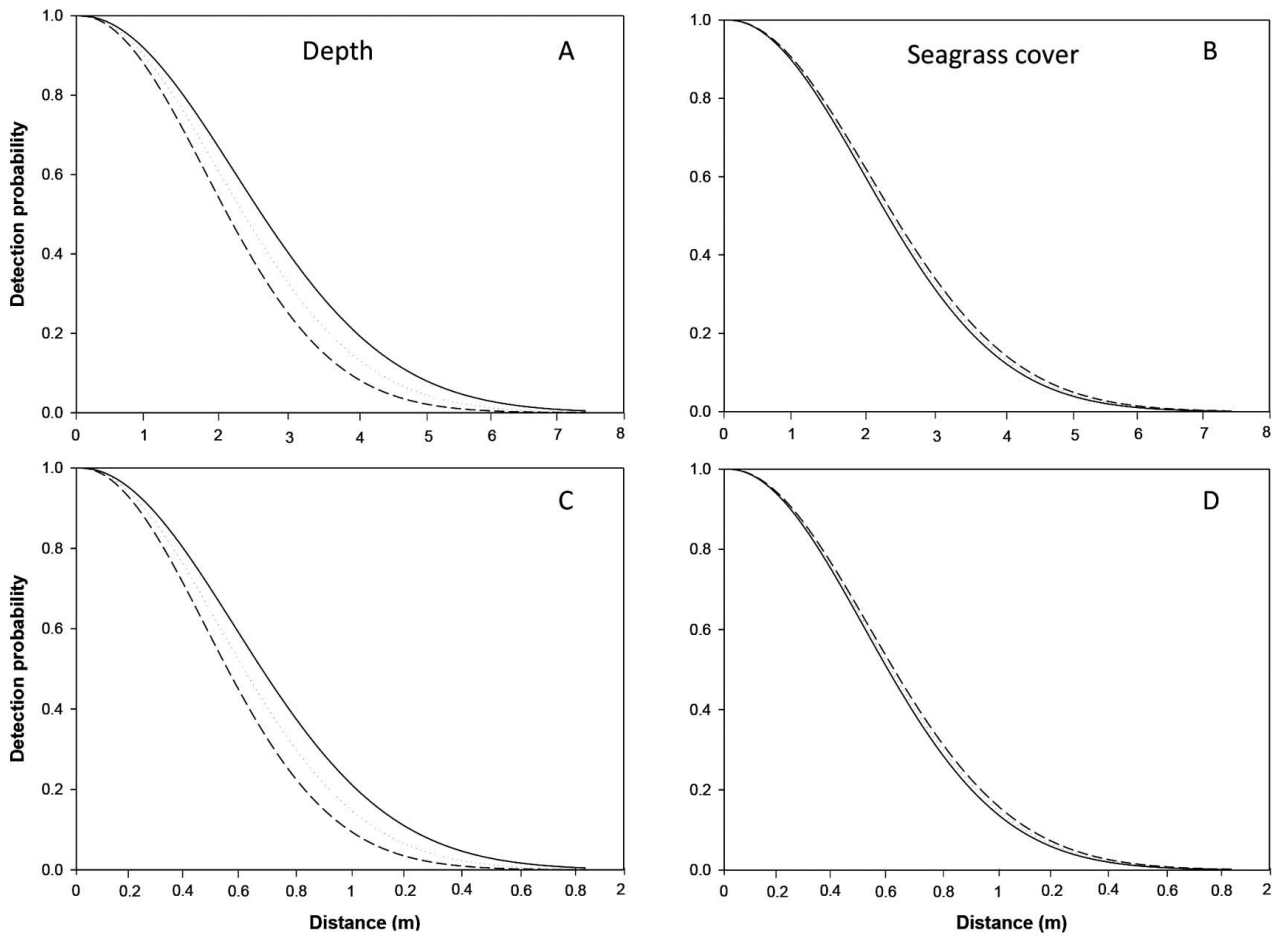


Fig. 6. – Effects of variability in depth and seagrass cover covariates on the detection probability for the GH (A-B) and the BH (C-D) models. For depth, lines indicate detection functions at 0.74, 0.81, and 0.89 m; for seagrass, detection functions at 30%, 60%, and 90% cover are shown. Transect half-width (i.e. maximum distances from the transect line) at 2 m and 7.9 m, respectively, for the BH and the GH are indicated.

Table 1. – Summary of areas, model-corrected population estimates and densities (ind./100 m²) in the entire BH and GH per depth class of 10 cm. See methods for further information about data sources.

Depth (cm)	Area (m ²)	BH		GH		
		no. ind.	Density (ind./100 m ²)	Area (m ²)	no. ind.	Density (ind./100 m ²)
20-29	18056.96	0	0	12796.52	42.21	0.18
30-39	198626.52	0	0	140761.74	1208.73	0.09
40-49	379196.09	0	0	268726.96	1745.94	1.35
50-59	469480.87	0	0	332709.57	1903.27	1.58
60-69	559765.65	565.89	0.05	396692.17	5387.47	1.44
70-79	487537.83	2875.08	0.28	345506.09	7554.54	4.69
80-89	451423.91	3803.02	0.40	319913.04	3085.13	7.10
90-99	325025.22	2816.41	0.41	230337.39	3044.20	4.03
100-109	252797.39	929.32	0.17	179151.30	2026.06	5.11
110-119	216683.48	1095.27	0.24	153558.26	42.21	3.97
120-129	288911.30	0	0	204744.35	0	0
130-139	343082.17	0	0	243133.91	0	0
Total		12084.99			78218.52	

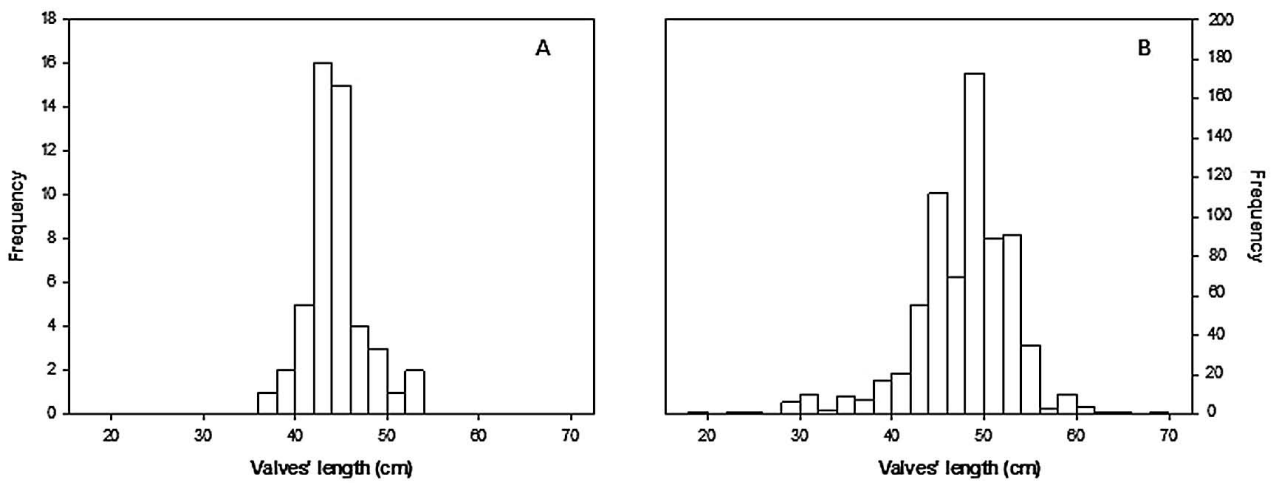


Fig. 7. – Proportion of individuals of each size (valve length) during shore-parallel transects at the Banya Sandspit in the BH (A) and in the GH (B).

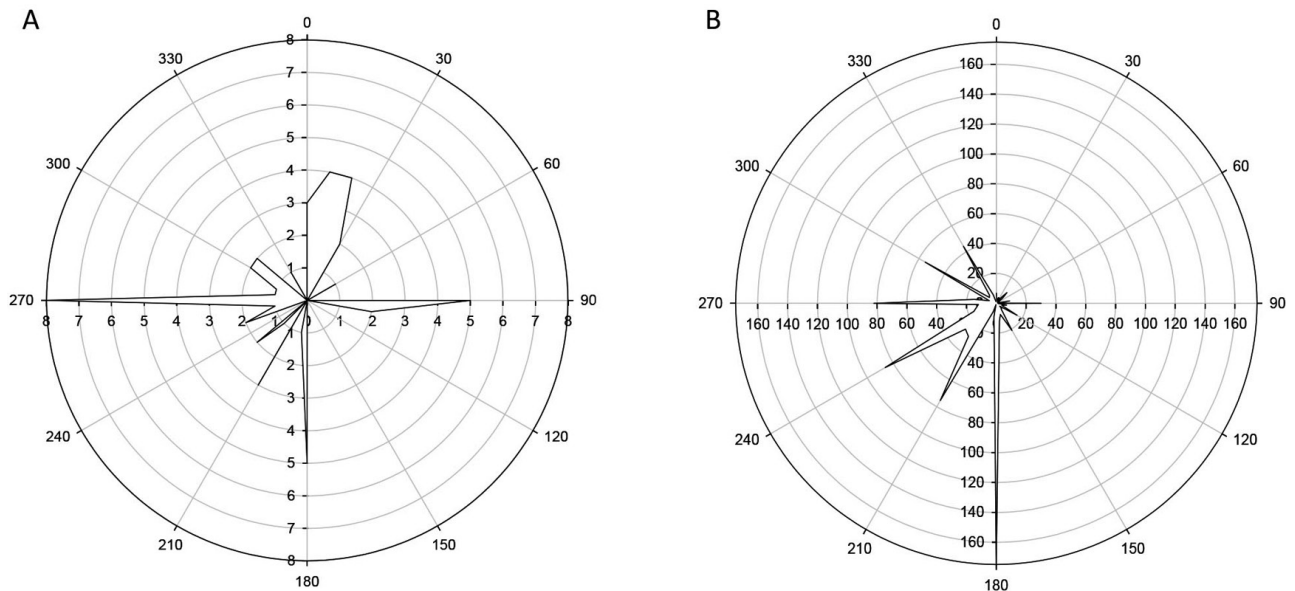


Fig. 8. – Number of *P. nobilis* individuals with valves oriented towards each geographical direction (0°-360°) in the BH (A) and in the GH (B).

by distance, and then by the depth covariate, possibly due to decreased visibility, whereas seagrass cover had little effect (Fig. 6a-d).

The model for the BH gave an estimation of 0.5 ind./100 m² and a total of 106.4 individuals within the

study area (i.e. line transects 2×2 m), whereas only 50 individuals were observed, so the underestimation was 53.01% in this region. In contrast, the model for the GH gave an estimated density of 3.6 ind./100 m² and a total of 3064.9 individuals within the study area (i.e.

line transects 2×7.9 m wide), which was much larger than the observed numbers (1018 ind.), so the underestimation was 66.75% (see also Fig. 6). These underestimation values were then added to raw estimates of individuals per depth interval to obtain a total estimate of 12084.99 individuals in the BH and 78218.52 individuals in the GH (see Table 1).

Population structure and valve orientation

A significant and moderately strong relationship was found between valve width and maximum length for the intact dead individuals found during the study ($R^2=0.486$; $df=21$; $F=7801.5$; $p<0.001$). Therefore, the width of individuals observed during the line transects (50 in the BH and 1022 in the GH, of which 720 individuals were measured) was converted into maximum valve length [$\ln W=1.2 \cdot (\ln L)^2 - 8.5 \cdot \ln L + 18.1$] and then the size distribution was investigated separately in the two regions. In the BH, the smallest individual recorded was 36.9 cm long and the largest 53.3 cm, with 64% of the population from 42-46 cm long (Fig. 7). In the GH, the size range was more than double (19.3 cm to 68.5 cm), with up to 70% of the individuals in the 45-55 cm interval (Fig. 7).

The geographical orientation (0° - 180°) of the valves showed different patterns in each region. In the BH, individuals were oriented in all geographic directions, with a larger frequency from E-W: 26.5%; Fig. 8). In contrast, in the GH the N-S and the NE-SW orientation were the dominant directions (25.3% and 35.7% of individuals, respectively; Fig. 8), coinciding with the direction of the main local winds, Mistral (N), 337° to 24° and Garbi (SW), 180° to 210° .

DISCUSSION

The population of *Pinna nobilis* in the Alfacs Bay was estimated to be 90303 individuals, the largest so far reported in shallow Mediterranean waters (<1.3 m) and comparable only to that reported by Katsanevakis and Thessalou-Legaki (2009) for a bay in Greece at a depth range of 4 to 22 m. Over 90% of the observed individuals were adults of 40 to 60 cm length, with no occurrence of juveniles <20 cm (see also Richardson et al. 1999, 2004) and indicative of a declining population. This is the first scientific report of *P. nobilis* in the Ebro Delta region, with the closest referenced populations located at the towns of L'Ametlla de Mar (ca. 35 km north from the Alfacs Bay) and Peñíscola (ca. 40 km south from the Alfacs Bay), both with bad perspectives for the conservation of the species (Guallart and Templado 2012). Although other studies have reported much higher local densities (up to >100 ind./100 m², Galinou-Mitsoudi et al. 2006) than those observed in Alfacs Bay, only three have investigated the abundance and the population structure of *P. nobilis* in areas larger than 10 ha (see Table 2). Given the similar adult sizes observed, the population in Alfacs Bay may endure recruitment gaps of several years that make it more susceptible to human activities and habitat alterations.

Table 2. – Population abundance and densities of *P. nobilis* (ind./100 m²) reported from the literature considering the geographical area investigated, the depth range and the type of habitat.

Location	Investigated area (m ²)	Depth range (m)	Total popul. (no. ind.)	Mean density (Max) (ind./100 m ²)	Habitat	Source
Souda Bay (Crete, Greece)	14715000	4-22	130900	0.89 (53)	<i>C. nodosa</i> / <i>C. racemosa</i>	Katsanevakis and Thessalou-Legaki (2009)
Alfacs Bay (Ebro Delta, Spain)	7096300	0.3-1.3	90303.52	1.61 (20)	<i>C. nodosa</i> sand	This study
Lake Vouliagmeni (Korinthiakos Gulf, Greece)	1504000	1-23	8501	0.57 (17)	mud	Katsanevakis (2006)
Chafarinas Islands (Morocco)	182000	7.4-11.2	NA	2.9 (NA)	<i>P. oceanical</i> / <i>C. nodosa</i> /macroalgae	Guallart and Templado (2010)
Gulf of Oristano (Sardinia, Italy)	67200	2-10	1285	2.7 (6.7)	<i>P. oceanical</i> / <i>C. nodosa</i>	Coppa et al. (2013)
Gulfs of Hammamet and Gabes (N and E coast, Tunisia)	33600	0-6	845	2.5 (20)	<i>P. oceanical</i> / <i>C. nodosa</i> /macroalgae	Rabaoui et al. (2008)
Gulfs of Hammamet and Gabes (E and SE coast, Tunisia)	21100	0-6	327	1.5 (56)	<i>P. oceanical</i> / <i>C. nodosa</i> /macroalgae	Rabaoui et al. (2010)
Ghar El Melh lagoon (Tunisia)	5024	0.3-0.5	152	3.01 (9.6)	<i>Ruppia</i> spp./ <i>N. noltii</i> / <i>C. nodosa</i> /sand	Zakhama-Sraieb et al. (2011)
Gulf of Oristano (Sardinia, Italy)	4500	2-8	530	6.3 (29)	<i>P. oceanical</i> / <i>C. nodosa</i>	Addis et al. (2009)
Mijet National Park (Croatia)	1928	3-15	180	10.9 (20)	<i>C. nodosa</i>	Siletic and Peharda (2003)
Columbres Islands (Castellon, Spain)	7 areas of 100-314	20-34	55	1.5 (16)	<i>C. nodosa</i>	García-March and Kersting (2006)
Moraira Bay (Alicante, Spain)	1314	6 and 13	20 and 103	6 and 10.3	<i>P. oceanica</i>	Galinou-Mitsoudi et al. (2006)
Thermaikos Gulf (Greece)	70	2-3	73	104 (130)	NA	Richardson et al. (1999)
Almeria coast (SE Spain)	323	2-17	55	17 (30)	<i>P. oceanica</i>	Sanchez-Lizaso JL (Person. Com)
Tabarca Island (Spain)	300	NA	12	1.33(3)	<i>P. oceanica</i>	Barberá-Cebrián et al. (1996)
Coast of Alicante (Spain)	3000	5-15	90	1.13 (5.6)	<i>P. oceanical</i> / <i>C. nodosa</i>	

Depth was a central factor influencing the abundance patterns of *P. nobilis* in Alfacs Bay, but it had no apparent effect on shell size. All individuals were found at depths of between 0.3 and 1.3 m, with a peak at 0.6 to 0.9 m and sharp declines afterwards. Although the depth range of *P. nobilis* may vary from 0.5 to 60 m (Butler et al. 1993), peaks at depths lower than 1 m are rarely reported (but see Zakhama-Sraieb et al. 2011). More commonly, depth distributions peak at ranges from 3 to 15 m (García-March et al. 2006, García-March and Kersting 2006, Katsanevakis 2007, Coppa et al. 2013) and may be explained by the need to minimize exposure to wave action, as also evidenced by increasing valve sizes at greater depths and shell orientations coincident with the exposure that minimizes hydrodynamic effects (García-March et al. 2007a,b). Although no depth effect was observed on the size distribution of *P. nobilis*, a greater proportion of individuals (56.7%) had valves oriented towards the dominant winds (Mistral, N-S, and Garbí, SW-NE), suggesting that this could also be an important factor shaping the structure of the population. At the shallower end of the distribution, the abundance of individuals may also be constrained by strong daily and seasonal variations in temperature (Palacin et al. 1991) falling above the thermal tolerance of the species at 38°C (Schlieper et al. 1960). At the greater depths, the disappearance of *P. nobilis* coincided with the depth limit of *Cymodocea nodosa* at 1.3-1.5 m. Seagrass beds have been considered to be the most suitable substrate for *P. nobilis*, since their rhizomes allow a complex connection between byssus filaments and the sediments (García-March 2005). In fact, although individuals were detected in both vegetated and unvegetated areas (see also Guallart and Templado 2012), higher abundances (by ca. 40%) were observed in areas with 80%-100% cover, thus suggesting that dense meadows may favour the highest abundances of individuals. Although *P. nobilis* can sometimes be found in muddy/silty sediments (e.g. Katsanevakis 2006), resuspension of fine sediments may interfere with filtration and/or affect the abundance of phytoplankton and zooplankton, important dietary components of medium and large fan shells (Davenport et al. 2011), and prevent the presence of individuals in areas under the influence of agricultural discharges. In addition, low oxygen conditions in muddy substrates such as those beyond the seagrass limit may also reduce the abundance of individuals (Centoducati et al. 2007). In contrast, salinity stress is unlikely since ranges of variation at the northern shore of the Bay are above (ca. 30-35 ppm, Garcés et al. 1999) reported stress values at 25 ppm (Schlieper et al. 1960). We hypothesize that habitat use is strongly determined by siltation and low oxygen conditions, which also determines seagrass presence; thus, prior to rice cultivation, *P. nobilis* could have been present in deeper areas in the Alfacs Bay, and even possibly in adjacent coastal lagoons connected to the bay.

The size structure of the *P. nobilis* population was homogeneous, with only 1.2% of individuals having sizes of 20-30 cm, and the majority (91.5%) being between 40 and 60 cm, which according to data from

other Mediterranean locations corresponds to ages of 2 to 4 and 3 to 9 years, respectively (Richardson et al. 1999, 2004). A size distribution mainly characterized by large individuals is not uncommon in seagrass meadows (Addis et al. 2009) and may be the result of the effects of several factors. On the one hand, the influence of shallow waters has been suggested to induce the development of dwarf individuals, reaching a size plateau at ages ≥ 5 years (García-March et al. 2007a). Therefore, given the narrow depth range observed for fan shell distribution (0.3 to 1.3 m), it could be possible that individuals in Alfacs Bay attain lower maximum sizes than those reported for other Mediterranean locations (Zavodnik et al. 1991, Richardson et al. 1999) and actually have a more heterogeneous age structure. On the other hand, the absence of juveniles ≤ 20 cm (Richardson et al. 1999), which usually occur in shallower areas which extend to sheltered inlets (Katsanevakis 2006), could be partly due to a negative effect of adults on recruitment, mostly because of ingestion of planktonic larvae during water filtration (Davenport et al. 2011) or to resource limitation in areas with high densities, as observed for other bivalve species (e.g. Peterson 1982). In addition, the survival of juvenile fan mussels can be significantly affected by the abundance of large potential predators such as decapods, gastropods and octopuses (García-March et al. 2007a, Katsanevakis and Thessalou-Legaki 2009), all of which are common in the Alfacs Bay. Altogether, the homogeneous size structure observed may include one or more successful recruitment events, but the absence of young sizes suggests gaps of multiple years between them, as indicated at high adult densities for other species of bivalves (Strayer and Malcom 2006).

Individuals of *P. nobilis* were mostly concentrated along the Banya inner shelf (few isolated individuals were also observed in the NE corner of the bay, in front of the Tancada lagoon) but two distinctive areas were observed with higher densities of individuals in the outer half of the sandspit closer to the opening with the sea (0.89 ind./100 m²) and lower densities in the inner half bordering the salt pans (0.14 ind./100 m²). Both habitats featured similar levels of seagrass cover and habitat features. The mechanism(s) mediating this distribution are not yet well-understood but the limit between the two zones was clearly marked by the end of the salt pan dikes, which possibly alter water circulation and turn-over in the area in front of them. Hence, in addition to persistent ineffective recruitment in the GH, hydrodynamic barriers such as dikes may alter further larval settlement and dispersal, and contribute to the very low effective recruitment observed within the bay. Overall, factors controlling planktonic and early benthic stages may constrict the abundance and size structure of the *P. nobilis* population in Alfacs Bay.

Several important implications for the conservation of the species emerge from the data presented here. Given the lack of awareness of the presence of *P. nobilis* in the area, there is no current regulation of boating activity in shallow waters around the Banya Sandspit and free access to high-density areas (mostly by local recreational fishermen) is causing damage

and mortality of individuals by boat propellers. For example, we observed 11 pulled up, 22 dead, and up to 54 damaged individuals, with obvious scars from boat propellers. In addition, a ferry operating during the summer may account for the absence of *P. nobilis* near its mooring area. Given that the Banya Sandspit is a Natural Park and a Natural 2000 area and that *P. nobilis* is protected by the Barcelona Convention and the Habitats Directive (Annex IV), it appears necessary to develop a management plan that includes actions such as prohibited motor entrance and better coastal surveillance, particularly during the summer period. Another important issue for a conservation agenda is habitat preservation. Although no cause-effect relationships were established for human pressures in the habitat, there is a worrying coincidence between recurrent inputs of freshwater, nutrients and organic matter from rice cultivation (Delgado 1987, Garcés et al. 1999) and the absence of *P. nobilis* in northern regions of the bay. Similarly, the occurrence of lower numbers of individuals in the vicinity of salt pan dikes (i.e. the BH) suggests possible alterations of water circulation, wave exposure, and/ or water temperature and requires further investigation.

To conclude, though the population of *P. nobilis* in the Alfacs Bay is the second largest reported in the Mediterranean, it is especially vulnerable to a variety of human impacts. While the shallow limit of the distribution may be controlled by water temperature and exposure to desiccation, the deep limit appears to be related to an abrupt change in habitat, from sandy sediment with patches of *C. nodosa* to silty unvegetated sediments. Given the isolated nature of the Alfacs Bay population, and the absence of young size-classes, it might be especially vulnerable to losses of individuals and an integrative combination of conservation actions (i.e. boating and touristic restrictions, habitat restoration, artificial recruitment, and scientific monitoring) seems to be the most appropriate managerial approach.

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