Spatial distribution, abundance and habitat use of the endemic Mediterranean fan mussel Pinna nobilis in Gera Gulf, Lesvos (Greece): comparison of design-based and model-based approaches

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http://dx.doi.org/10.12681/mms.14156
Spatial distribution, abundance and habitat use of the endemic Mediterranean fan mussel *Pinna nobilis* in Gera Gulf, Lesvos (Greece): comparison of design-based and model-based approaches

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Handling Editor: Emma Cebrian

Received: 5 July 2017; Accepted: 6 August 2018; Published on line: 31 December 2018

Abstract

An important population of the endemic Mediterranean fan mussel *Pinna nobilis* thrives in the marine protected area of Gera Gulf (Lesvos island, north-eastern Aegean Sea, Greece), and was assessed for the first time. To estimate the abundance, spatial distribution and habitat use of fan mussels in Gera Gulf, a distance sampling underwater survey was conducted. Detectability was modelled to secure unbiased estimates of population density. Two approaches were applied to analyze survey data, a design-based and a model-based approach using generalized additive models. The first approach was based on stratified random sampling on two strata, an assumed ‘preferable’ zone close to the coastline and an assumed unsuitable habitat, with predominantly muddy sediments, in which low sampling effort was applied. For the needs of the model-based approach, a dedicated cruise was conducted to collect bathymetric data with a single-beam echo-sounder and map the bathymetry of the study area. A very high-resolution image from the Worldview-3 satellite was processed, based on an object-based image analysis, for mapping all main habitat types in the study area. The estimated abundance using the design-based approach was low-biased as the stratum of pre-assumed unsuitable habitat proved to include patches of suitable habitats with high population densities that were missed by sampling. The model-based approach provided an abundance estimate of 213300 individuals (95% confidence interval between 97600-466000 individuals), which renders the fan mussel population of Gera Gulf the largest recorded population in Greece. Population density peaked between 1.5-8 m depth and became practically zero at depths >15 m. A bathymetric segregation of fan mussel size classes was noted, with the density of small individuals peaking in shallow waters, while that of large individuals peaked deeper. The highest population densities were observed in *Posidonia oceanica* meadows, followed by mixed bottoms (with reefs, rocks and sandy patches), while densities were very low on sandy and zero on muddy sediments. The current assessment provides a baseline for future monitoring of the fan mussel population in Gera Gulf. In view of the current (2017-2018) ongoing mass mortality of the species in the western Mediterranean, continuous monitoring of the main fan mussel populations, such as the one in Gera Gulf, is of utmost importance.

Keywords: Abundance estimation; spatial distribution; distance sampling; line transects; SCUBA; endangered species.

Introduction

*Pinna nobilis* Linnaeus, 1758 is a marine bivalve mollusc of the Pinnidae family, commonly known as noble pen shell or fan mussel. It is one of the largest bivalves in the world and the largest in the Mediterranean Sea, where it is endemic. The average anterio-posterior length of adult individuals is 30-50 cm but it can reach the size of 120 cm (Zavodnik et al., 1991). Its lifespan commonly exceeds 20 years and can even reach 45 years (Rouanet et al., 2015). *P. nobilis* occurs at depths ranging between 0.5 and 60 m. It usually inhabits seagrass meadows such as *Posidonia oceanica*, *Zostera marina*, *Z. noltii* and *Cymodocea nodosa* (Zavodnik et al., 1991), but it can also be abundant in macroalgal beds (Katsanevakis & Thessalou-Legaki, 2009) and unvegetated soft bottoms (Katsanevakis, 2006; Addis et al., 2009).

*P. nobilis* was formerly targeted for its meat and byssus from which sea silk was produced. It is currently strictly protected under the EU Habitats Directive (92/43/EEC, Annex IV), the Protocol for Specially Protected Areas and Biological Diversity in the Mediterranean of the Barcelona Convention (Annex II), and the national legislation of most Mediterranean countries. Nevertheless, it is still illegally exploited and marketed in many countries (Katsanevakis et al., 2011). Despite protection, in the last decades its populations have been declining (Basso et al., 2015), due to direct threats such as trawling...
and anchoring (Vázquez-Luis et al., 2015), illegal collection by divers for food, decorative purposes, and for its byssus (Zavodnik et al., 1991; Katsanevakis, 2007a), and indirect threats such as habitat loss or degradation. Since autumn 2016, a mass mortality event, caused by the parasite Haplosporidium pinnae (Catanese et al., 2018) has caused, so far, an estimated loss of ~90% of the Spanish P. nobilis populations (Vázquez-Luis et al., 2017) and has raised concerns about the status of the species in the entire Mediterranean basin.

In addition to the general prohibition on its exploitation and marketing, the NATURA 2000 network can contribute to the protection of important populations of P. nobilis and its important habitats, such as Posidonia oceanica meadows. NATURA 2000 is one of the world’s most extensive networks of conservation areas, which currently consists of more than 27,200 sites, of which approximately 15% include marine areas (Mazaris et al., 2018). Nevertheless, many of the marine sites of the NATURA 2000 network are poorly monitored and managed, and proper assessments of the population status of protected species within their boundaries are often lacking.

The aim of this study was to estimate the population status of an important Pinna nobilis population in Gera Gulf (Lesvos island, Greece), which is part of the NATURA 2000 network (site codes: GR4110013, GR4110005). Two approaches were followed for abundance estimation, a design-based and a model-based approach. The latter also allowed assessment of the spatial distribution of the species in the gulf and its habitat use, i.e. variability in its abundance in different habitat types, which is actually a combination of preferential settlement and differential mortality. Despite Gera Gulf being part of the NATURA 2000 network, there have been no previous assessments of its P. nobilis population, and thus this study serves as a baseline for assessment of future population trends.

In view of the ongoing mass mortality of the species in the western Mediterranean (Vázquez-Luis et al., 2017), monitoring all important populations of the species is of utmost importance.

Methods

Study Area

Gera Gulf is an enclosed elongated embayment, located in the south-eastern part of Lesvos Island, north-eastern Aegean Sea, Greece (Fig. 1). It receives discharges from seasonal streams and small rivers, and is connected to the open sea through a narrow channel of ~6.5 km length and 300 – 800 m width. For the purposes of this study, a detailed large scale map of Gera Gulf was created using a SENTINEL-2 satellite image. The total surface of the gulf is 4009.9 ha (calculated using ArcGIS 10.2.2 ‘calculate geometry function’). Nearshore the substrate is dominated by sand mixed with gravel, cobbles or rocks, followed further offshore by sandy and muddy mixtures. In the south and western part of the gulf, there are patchy Posidonia oceanica meadows.

Bathymetry

Bathymetric data were collected during a cruise onboard R/V Amfitriti, using a Simrad CA44 single-beam echo-sounder operating at 200 kHz, along a ~270 km survey grid of crossing lines. Vessel speed was maintained at about 4 knots. The depth was corrected for sound velocity (1500 m/s) and transducer depth. ArcGIS 10.2 was used to produce the bathymetry of the gulf through interpolation. However, it is well-known that different interpolation techniques produce different values at the same grid points thus introducing a degree of uncertainty (Chiles & Delfiner, 1999). Therefore, to adopt the most reliable results, 4 interpolation methods were examined: Topo to raster, Kriging (Ordinary and Universal), Inverse Distance Weighted (with topical and spherical parameters) and Spline with barriers. Errors quantification was managed by the Mean Absolute Error (MAE) and the Root Mean Square Error (RMSE). For the validation proce-
A subgroup of 13,830 points was pre-selected (25% of the total points) to compare the results with the initial dataset and estimate the MAE and RMSE. The comparison showed that the Spline method was the best, having the lowest MAE (0.02) and RMSE (0.14). Finally, a raster file with 2-m pixel size was created from the point data set.

**Habitat Map**

A habitat map of Gera Gulf was created by classifying a very high spatial resolution image from the Worldview-3 satellite, acquired on 18-11-2015. The spatial resolution of the five multispectral bands (coastal, blue, green, red, infrared) was 1.5 m. The image was pre-processed by applying a land mask derived from the infrared band. An Object Based Image Analysis (OBIA) approach was followed, using the other four bands and eCognition 5.4. software. The analysis involved image segmentation into small objects (segments), which are groups of pixels with similar characteristics, used as the main processing element (Blaschke et al., 2010). Segmentation was applied using a scale factor of 50, and a homogeneity criterion (with shape value of 0.1 and compactness value of 0.5). Finally, supervised classification took place in the following four classes: (a) *Posidonia oceanica* meadows, (b) mixed sea bed (cobbles, rocky reefs and sandy patches), (c) sandy sediment and (d) muddy sediment. There were no extensive rocky areas in Gera Gulf and all hard substrates were patchily distributed among soft substrates, which was the reason for not including a separate hard substrate habitat in our classification.

**Line transect sampling – Field Work**

The single observer line transect distance sampling method by SCUBA diving was applied for abundance estimations (Katsanevakis, 2007b). This approach has been used extensively for surveying *Pinna nobilis* populations (Katsanevakis, 2006, 2007b; Katsanevakis & Thessalou-Legaki, 2009) and is better compared to strip transect sampling, as detectability is properly accounted for. The critical assumption of strip transects is that all individuals present within the transect surface are detected. However, this assumption can easily be violated in the marine environment leading to substantial underestimation of population density and abundance (Katsanevakis et al., 2012). The imperfect detectability issue is overcome in line transect sampling, where a standardized survey is conducted along a series of lines searching for the animals of interest. For each animal detected, the distance, y, from the line or point is recorded. A detection function, \( g(y) \), is fitted from the set of recorded distances (Buckland et al., 2001, 2004), which is used to estimate the proportion of animals missed by the survey and, hence, accurately estimate abundance.

Eighteen transect locations were randomly placed in the study area, 15 close to the shore and three in the central part of the gulf. Sampling was conducted in the summer of 2016. Sampling effort was focused in the shallow coastal areas, as preliminary surveys indicated the absence of fan mussels in the deeper muddy seabed. Nearshore transects were defined vertically to the coast, and oriented towards the centre of the gulf using a diving compass. Transect length varied between 100 and 200 m, depending on the depth, diving conditions and diving limitations. Each transect length \( L \) was defined with a nylon line deployed using a diving reel. The line was segmented at five meter intervals (hereafter called segments) with water resistant labels, and was marked with water resistant paint at one-meter intervals. Depth measurements were taken at the mid-point of each segment with a dive computer. The habitat type was classified into four basic categories (sandy, muddy, mixed, and *Posidonia oceanica* meadows) and the dominant category of each segment was recorded. For each fan mussel observation, the following data were noted on diving slates: the longitudinal distance from the start of the transect \( l \), the perpendicular distance from the line \( y \) and shell size \( S \), defined as the maximum dorso-ventral length of the shell. The perpendicular distances were measured with a measuring tape (0.5 cm accuracy) and shell size with vernier callipers (for widths >15 cm with an accuracy of 0.5 cm and for widths <15 cm with an accuracy of 0.05 cm). For each transect, a visibility index was estimated empirically: one of the two divers stood still while holding a white board and the start of a measuring tape, while the other receded slowly. When the board was barely visible, the corresponding distance was considered as an index of average visibility.

**Detection function modelling**

Two candidate models for the detection function, \( g(y) \), were fitted, the one-parameter half-normal model

\[
g(y) = \exp\left(\frac{-y^2}{2\sigma^2}\right)
\]

and the two-parameter hazard-rate model

\[
g(y) = 1 - \exp\left(\frac{-y}{\sigma}\right)^b
\]

where \( \sigma \) is a scale parameter and \( b \) a shape parameter (Buckland et al., 2001). It is possible to include covariates \( v_i \) in these models, i.e. variables that may affect detectability, through the scale parameter \( \sigma \), according to the equation:

\[
\sigma = \exp(\beta_0 + \beta_1 v_1 + \beta_2 v_2 + \cdots + \beta_i v_i)
\]

where \( \beta_i \) are estimable parameters (Marques & Buckland, 2004).

In this study, three covariates were considered as potentially affecting detectability, namely, the size of fan mussel individuals, habitat type and water visibility. The hazard-rate and half-normal models were used with no, one, two or three covariates. Thus, sixteen candidate models \( m_i \) (\( i = 1 \) to 16) were included in the set of candidate models for the detection function (Table 1). In models with an odd index, the half normal function was used,
whereas the hazard rate function was used in those with an even index. In models $m_1$ and $m_2$, the $\sigma$ parameter was constant, while in the rest at least one covariate was included.

The best model was selected using Akaike’s Information Criterion (AIC; Akaike, 1973). Goodness-of-fit of the best model was assessed with Q-Q plots and the Cramér-von-Mises test, weighted to give higher weight to distances near zero (Burnham et al., 2004). The Multiple Covariates Distance Sampling (MCDS) engine in DISTANCE v7.0 (Thomas et al., 2010) was used for detection function modelling.

**Design-based approach for abundance estimation**

In the design-based approach, inference was based on the design characteristics of the survey, i.e. stratified random sampling, and each transect was treated as a sampling unit. Two strata were defined based on preliminary observations that fan mussels were mostly restricted to the nearshore zone. Towards the deeper part of the gulf,

Table 1. Parametrization of the 16 candidate models $m_i$ for the detection function, average probability of detection $P_a$ (± SE), Akaike differences $\Delta_i$, estimated population density and abundance of *P. nobilis* in the study area, and 95% confidence intervals of abundance (based on bootstrapping; 999 resamples). The best model is given in bold.

<table>
<thead>
<tr>
<th>model</th>
<th>function</th>
<th>covariate</th>
<th>No. of parameters</th>
<th>$P_a$</th>
<th>$\Delta_i$</th>
<th>population density</th>
<th>abundance</th>
<th>95% CI of abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m_1$</td>
<td>Half-normal</td>
<td>-</td>
<td>1</td>
<td>0.580 ± 0.070</td>
<td>15.19</td>
<td>0.0028</td>
<td>112800</td>
<td>64200-198400</td>
</tr>
<tr>
<td>$m_2$</td>
<td>Hazard-rate</td>
<td>-</td>
<td>2</td>
<td>0.740 ± 0.060</td>
<td>17.98</td>
<td>0.0022</td>
<td>88900</td>
<td>50900-155300</td>
</tr>
<tr>
<td>$m_3$</td>
<td>Half-normal</td>
<td>size</td>
<td>2</td>
<td>0.585 ± 0.055</td>
<td>16.98</td>
<td>0.0029</td>
<td>112900</td>
<td>64500-197500</td>
</tr>
<tr>
<td>$m_4$</td>
<td>Hazard-rate</td>
<td>size</td>
<td>3</td>
<td>0.725 ± 0.050</td>
<td>20.34</td>
<td>0.0024</td>
<td>90500</td>
<td>51800-158000</td>
</tr>
<tr>
<td>$m_5$</td>
<td>Half-normal</td>
<td>visibility</td>
<td>2</td>
<td>0.575 ± 0.055</td>
<td>12.67</td>
<td>0.0029</td>
<td>114300</td>
<td>65300-199900</td>
</tr>
<tr>
<td>$m_6$</td>
<td>Hazard-rate</td>
<td>visibility</td>
<td>3</td>
<td>0.690 ± 0.055</td>
<td>16.99</td>
<td>0.0024</td>
<td>95400</td>
<td>54600-166600</td>
</tr>
<tr>
<td>$m_7$</td>
<td>Half-normal</td>
<td>habitat</td>
<td>3</td>
<td>0.560 ± 0.060</td>
<td>2.31</td>
<td>0.0030</td>
<td>118000</td>
<td>67300-206700</td>
</tr>
<tr>
<td>$m_8$</td>
<td>Hazard-rate</td>
<td>habitat</td>
<td>4</td>
<td>0.695 ± 0.055</td>
<td>6.76</td>
<td>0.0025</td>
<td>94500</td>
<td>54100-165200</td>
</tr>
<tr>
<td>$m_9$</td>
<td>Half-normal</td>
<td>visibility &amp; size</td>
<td>3</td>
<td>0.575 ± 0.055</td>
<td>14.38</td>
<td>0.0029</td>
<td>114300</td>
<td>65300-200000</td>
</tr>
<tr>
<td>$m_{10}$</td>
<td>Hazard-rate</td>
<td>visibility &amp; size</td>
<td>4</td>
<td>0.690 ± 0.055</td>
<td>18.89</td>
<td>0.0024</td>
<td>95600</td>
<td>54700-167100</td>
</tr>
<tr>
<td>$m_{11}$</td>
<td>Half-normal</td>
<td>habitat &amp; size</td>
<td>4</td>
<td>0.550 ± 0.050</td>
<td>2.17</td>
<td>0.0030</td>
<td>119100</td>
<td>68000-208700</td>
</tr>
<tr>
<td>$m_{12}$</td>
<td>Hazard-rate</td>
<td>habitat &amp; size</td>
<td>5</td>
<td>0.700 ± 0.055</td>
<td>9.34</td>
<td>0.0025</td>
<td>93800</td>
<td>53700-163800</td>
</tr>
<tr>
<td>$m_{13}$</td>
<td>Half-normal</td>
<td>habitat &amp; visibility</td>
<td>4</td>
<td>0.550 ± 0.050</td>
<td>0.00</td>
<td>0.0030</td>
<td>120100</td>
<td>68500-210400</td>
</tr>
<tr>
<td>$m_{14}$</td>
<td>Hazard-rate</td>
<td>habitat &amp; visibility</td>
<td>5</td>
<td>0.720 ± 0.060</td>
<td>10.29</td>
<td>0.0025</td>
<td>91500</td>
<td>52400-159800</td>
</tr>
<tr>
<td>$m_{15}$</td>
<td>Half-normal</td>
<td>Habitat &amp; size &amp; visibility</td>
<td>5</td>
<td>0.545 ± 0.055</td>
<td>0.73</td>
<td>0.0031</td>
<td>120900</td>
<td>69000-211900</td>
</tr>
<tr>
<td>$m_{16}$</td>
<td>Hazard-rate</td>
<td>Habitat &amp; size &amp; visibility</td>
<td>6</td>
<td>0.695 ± 0.060</td>
<td>6.86</td>
<td>0.0026</td>
<td>94800</td>
<td>54200-165800</td>
</tr>
</tbody>
</table>
muddy sediments prevail and the substrate is unsuitable for the survival of fan mussels. It has been extensively documented that fan mussels are absent in muddy sediments (e.g. Katsanevakis, 2006). Hence, the first stratum was defined as a 200-m buffer zone along the coastline, while the second stratum included all the rest of the gulf (Fig. 2). As zero densities were anticipated in the second stratum, assuming that only muddy sediments occur, the survey effort was relatively low, also due to logistical constraints (need for support vessel, costs). In total, 15 transects were randomly defined in the first stratum and three transects in the second stratum. ArcMAP v10.2.2 was used to define the two strata and estimate their areas ($A_1, A_2$).

The total number of fan mussels within the covered transects was estimated through the Horvitz-Thompson-like estimator (Borchers, 1996) $\hat{n}_{ct} = \sum_{j=1}^{n} \frac{1}{\hat{p}_j}$, where $\hat{p}_j$ is the probability of detecting individual $j$, and was obtained from the estimated best model of the detection function. Hence, the population density at each stratum $h$ ($h = 1, 2$) was estimated as $\hat{D}_h = \frac{\hat{n}_{ct,h}}{A_{ct,h}}$, where $A_{ct,h}$ is the surface of the covered transects in stratum $h$. The standard error of the population density at each stratum was estimated as $SE_{D_h} = \sqrt{\frac{\sum (D_i - \hat{D}_h)^2}{n(n-1)}}$, where $D_i$ is the estimated density at each transect. The overall population density in the entire study area was estimated as $\hat{D}_{tot} = (A_1 D_1 + A_2 D_2)/A$, where $A = A_1 + A_2$ is the total study area. Total abundance was estimated as $\hat{N}_{tot} = A \hat{D}_{tot}$, and the corresponding standard error was obtained from the equation $SE_{R_{tot}} = A \sqrt{W_1^2 SE_{D_1}^2 + W_2^2 SE_{D_2}^2}$, where $W_i = A_i / A$ (Krebs, 1999).

**Model-based approach for estimating abundance, spatial distribution and habitat use**

The second method applied for abundance estimation was a model-based approach, as described by Katsanevakis (2007b) and Katsanevakis & Thessalou-Legaki (2009). Specifically, the count method of Hedley & Buckland (2004) was applied; according to this method, the transect lines are divided into smaller discrete units called segments (of 5-m length), and the estimated number of individuals in each segment is modelled by a Generalized Additive Model (GAM; Hastie & Tibshirani 1990) using explanatory spatial covariates. The Density Surface Modelling (DSM) engine in DISTANCE v7.0 (Thomas et al., 2010) was used for the model-based analysis.

Specifically, the total number of individuals within each segment $i$ was estimated using the Horvitz-Thompson-like estimator $\hat{r}_i = \sum_{j=1}^{n} \frac{1}{\hat{p}_{ij}}$ (Hedley et al., 2004), where $\hat{p}_{ij}$ was obtained from the best model of the detection function. These estimated values of abundance in each segment were related to spatial covariates using the general GAM formulation

$$f(E[\hat{r}_i]/A_s) = c + \sum_m s_m(x_m) + \sum_F F_{r_F}$$

where $f$ is the link function, $c$ is the intercept, $s_m(.)$ is the 1-dimensional smooth function for the predictor variable $m$, $x_m$ is the value of predictor variable $m$ for segment $i$, $F_{r_F}$ are the categorical predictors, and $A_s$ is the covered area of the segment.

For this study, two spatial covariates were used: habitat type as a categorical variable and depth as a continuous variable. Both are considered very important for predicting *P. nobilis* population density (Katsanevakis, 2007b; Katsanevakis & Thessalou-Legaki, 2009). A quasi-poison distribution and logarithmic link were used. The latter ensures positive values for the mean response. The smooth function $s(.)$ was represented using cubic regression splines, estimated by penalized iterative least squares (Wood, 2006). Four different GAM models were created; $h_i$ with no predictor, $h_i$ with habitat type as predictor, $h_i$ with depth as predictor, and $h_i$ with both hab-
itat type and depth as predictors. The best GAM model was chosen according to the generalized cross validation (GCV) score (Wood, 2006). For this analysis, the DSM and MRDS engines in DISTANCE v7.0 (Thomas, et al. 2010) and the package 'mgcv' (Wood, 2000, 2006) in R v3.3.3 (R Core Team, 2015) were used.

For abundance predictions, the study area was segmented into 64152 cells, measuring 25 x 25 m. For each cell, the average depth and dominant habitat type were estimated, according to the bathymetric and habitat maps. For each cell, the abundance of fan mussels $E[\hat{n}]$ was predicted using the best GAM model. The total abundance of $P. nobilis$ in Gera Gulf was estimated as the sum of the predictions for all cells, i.e. $\hat{N} = \sum E[\hat{n}]$. These predictions were imported and visualized in a density surface map of Gera Gulf using ArcMap v10.2.2.

Total variance was estimated by applying the delta method (Seber, 1982), according to the equation $[cv(\hat{N})]^2 = [cv(\hat{p})]^2 + [cv(\hat{n}_{DSM})]^2$, where $cv(\hat{p})$ is the coefficient of variation of the estimator of detection probability and $cv(\hat{n}_{DSM})$ is the coefficient of variation related to DSM. The first component was estimated empirically (Buckland et al., 2001), while for the second one a nonparametric bootstrap approach was followed, as described in Katsanevakis & Thessalou (2009). No autocorrelation was evident in the variogram of the deviance residuals and thus the 5-m segment was selected as the sampling unit for the bootstrapping.

**Results**

**Bathymetry and habitat mapping**

The gulf has a maximum depth of 19 m (29 m at the channel) and is characterized by a relatively smooth morphology down to ~11-12 m water depth (Fig. 3). The steeper slope inclinations are encountered towards the southeast, whereas the smoother relief appears at the NNW side of the gulf. Between the ~12 and 19 m isobaths a peculiar microrelief occupies the seafloor in the form of small hummocks that are distributed almost uniformly around the gulf. Their maximum height reaches 2 m in the south, close to the channel connecting Gera Gulf to the open sea.

The analyzed satellite image enabled mapping of the benthic habitats in the entire study area (Fig. 3), thanks to the shallow depth, relatively transparent waters on the day of acquisition, and very high image resolution. Image classification allowed identification of the areas with *Posidonia oceanica* meadows (a total area of 1.21 km²), with a large meadow in the south-western part of the gulf, narrow zones (0.10 km²) of mixed bottoms at various locations along the coastline, and extensive areas of sandy (13.65 km²) and muddy (24.83 km²) sediments, the latter covering the central part of the gulf.

**Detection function modelling – design-based approach for abundance estimation**

The total sampling effort (i.e. total length of the transects) was 2800 m. Overall, 194 fan mussel individuals
were recorded at distances of up to 5.12 m from the transect line. No individual was found in stratum 2. Their size (maximum width) varied between 3.97 and 19.65 cm and had a bimodal distribution (Fig. 4). There was an apparent segregation of size classes. Small individuals peaked in shallow waters, while large individuals were less common in the shallow zone and peaked in the depth zone of 3.6-4.8 m (Fig. 4). Visibility varied between 1.5 and 7.0 m. Data were right-truncated at 4.2 m to avoid the effect of outliers [and thus the covered area of each segment was 5 m x (4.2 m x 2) = 42 m²].

Based on AIC, model \( m_{13} \) (half-normal with visibility and habitat type as covariates) was the best amongst all candidate models (Table 1). This model gave a good Q-Q plot and provided a good absolute fit (Cramér-von Mises test; \( p = 0.75 \)). Model \( m_{15} \), which included visibility, habitat type and size as covariates, was also substantially supported by the data (\( \Delta_7 = 0.73 \)) and produced a very similar estimate of abundance (Table 1). The best model (\( m_{13} \)) is given by the equation:

\[
g(y) = \exp \left( -\frac{y^2}{2 \times [101.7 \times \exp((0.08923 \cdot \text{visibility}) + 0.4765 \cdot \text{mixed} + 0.8425 \cdot \text{Posidonia})]^2} \right)
\]

where the distance from the line, \( y \), is in cm, ‘visibility’ in m, and the two variables ‘mixed’ and ‘Posidonia’ are 1 (one), if the habitat is mixed or *Posidonia oceanica* meadow respectively, and zero otherwise (Fig. 5).

Surprisingly, the detectability of fan mussels in *Posidonia oceanica* meadows was better than the detectability in mixed or sandy sediments (Fig. 5). This was largely because of the within-transect variation of visibility. The estimated index of visibility was an estimated average for the wider area on the specific date. However, at different habitats within each transect, visibility varied substantially. In *Posidonia oceanica* meadows, visibility was better than in sandy areas where currents and the movement of divers increased turbidity through resuspension of fine sediment particles, and thus detectability was generally low. Based on \( m_{13} \) and the design-based approach, *Pinna nobilis* abundance in Gera Gulf was estimated to be \( \hat{N} = 120100 \), with a 95% confidence interval of 68500–210400 individuals, exclusively in Stratum 1.

**Density surface models – GAMs**

According to the GSV score, \( h_i \) that included both depth and habitat type as predictor variables, was the best model for DSM (Table 2). The expression of \( h_i \) is \( f(E[\hat{N}_i]/A_i) = c + s(d) + F(H) \), where \( c (\pm SE) = -4.50 (\pm 0.59) \), \( A_i = 42 \text{ m}^2 \), while the smooth function for depth \( s(d) \) and the categorical predictor \( F(H) \) are given in Fig. 6. Population density was
higher in shallow waters between 1.5 and 8 m depth, declined in very shallow areas <1.5 m or at depths >8 m, and became practically zero at depths >15 m. The highest population densities were observed in *Posidonia oceanica* meadows, followed by mixed bottoms, while densities were very low on sandy and zero on muddy sediments (Fig. 6).

Based on $h_4$, *Pinna nobilis* abundance in Gera Gulf was estimated to be $\hat{N} = 213300$, with a 95% confidence interval between 97600–466000 individuals. Moreover, a density surface map of Gera Gulf (Fig. 7) was produced based on $h_4$. In the south-eastern part of the study area, fan mussels were restricted to a very narrow nearshore zone, whereas in the western part of the study area fan mussels were distributed throughout a much wider zone (Fig. 7). The highest predicted densities coincided with the *P. oceanica* patches.

### Discussion

**Comparison of the two approaches**

The point estimate of abundance of *P. nobilis* in Gera Gulf using the design-based approach was much lower than the point estimate using the model-based approach. The main reason for this discrepancy was that the initial assumption of the design-based approach – that in the entire area beyond the 200-m buffer zone muddy sediments prevail and thus the substrate is inappropriate for fan mussels – is not true. In fact, as revealed by habitat mapping, the non-muddy area used in the model-based approach is almost double the size of the non-muddy area assumed in the design-based approach (Stratum 1). Hence, there were substantial areas suitable for *P. nobilis* in Stratum 2, which were missed by the limited sampling effort in that stratum, thus resulting in substantial underestimation of abundance.

Due to logistical constraints, habitat mapping was not
available before survey design. Otherwise, the distribution of the main habitat types would have been used as the basis for stratification. In the absence of habitat mapping, the initial observation that *P. nobilis* is restricted nearshore was used as the basis for stratification. There are many potential advantages of stratification of the study area based on subjective information and previous knowledge, such as reducing the survey cost and uncertainty in the estimates (Krebs, 1999; Morrison et al., 2001). In our case, this has been proved to be a problematic approach as Stratum 2 was under-sampled, due to our belief of zero abundance, and the related fan mussel population was underestimated. When dealing with sparsely distributed individuals over large areas, it is not uncommon to find a larger proportion of the population in the "low-density" stratum than in the "high-density" strata or "preferred" areas, as the low density is often multiplied by a huge area (McDonald, 2004).

The model-based approach was advantageous not only for making more accurate abundance estimates but also because it provides additional information on the spatial distribution of the species and its habitat use. The precision of the abundance estimate by the model-based approach could be greatly improved by stratifying the study area according to the habitat types. In that respect, this study can serve as a baseline for future monitoring of the species and for improving sampling design.

**Effect of depth and habitat type on the distribution of fan mussels**

When analyzing shelf assemblages, depth is the main gradient along which faunal changes occur (e.g. Bianchi, 1992; Demestre et al., 2000; Katsanevakis et al., 2009). This is less due to a direct effect of depth (because of the increase of pressure) but mostly due to the correlation of depth with many crucial environmental parameters such as bottom substratum, hydrodynamics, light intensity, temperature, primary and secondary productivity.

The pattern of bathymetric variation of fan mussel density found in this study was that of high densities at depths between 1.5 and 8m, lower densities < 1.5m or > 8m, and zero densities below 15 m. Similar results have been found in other studies, although density peaked deeper and, overall, the density-depth curve shifted at higher depths. Such data are available for two other areas in Greece, namely, Lake Vouliagmeni (Katsanevakis, 2007b) and Souda Bay (Katsanevakis & Thessalou-Legaki, 2009). In Lake Vouliagmeni, there was a main peak of population density at depths of 12–13 m, reduced density in very shallow waters, and practically zero densities at depths >22 m (Katsanevakis, 2007b). In Souda Bay, there was a density peak at a depth of ~15 m and practically zero densities in shallow areas (<4 m depth) and at depths >30 m. In the Cabrera National Park (Balearic Islands, Spain), the density peak was found at 9 m (Vázquez-Luís et al., 2014) and although density declined with depth,
fan mussels were found even at 46 m. In Tunisia, in a study conducted at a depth range of 0 to 6 m, Rabaoui et al. (2010) predicted a density of practically zero at 0.3 m depth, increasing with depth; in the absence of deeper transects, the depth of the peak was unknown. These differences in the bathymetric distribution of the species among studies are due to the local conditions of each area.

Two main factors seem to restrict fan mussel populations in very shallow waters, wave action (García-March et al., 2007a) and poaching by free divers (Katsanevakis 2007a). According to García-March et al. (2007), wave action causes increased mortality and chronic levels of hydrodynamic stress, which substantially decreases with depth, and thus the selective pressure on the population is the highest in very shallow waters. In addition, poaching by free divers causes a selectively higher mortality in shallow waters, especially for large individuals, which may greatly affect fan mussel densities and the structure of the population (Katsanevakis, 2007a). Poaching on fan mussels can be severe, greatly affecting their population dynamics and causing a size segregation of individuals, with larger and older individuals restricted to deeper areas and smaller and younger individuals dominating in shallow waters (Katsanevakis, 2009). These factors have probably contributed to the size segregation of fan mussels observed in Gera Gulf.

*P. nobilis* was absent from the deeper muddy bottom of Gera Gulf; this is in agreement with the studies in Lake Vouliagmeni (Katsanevakis, 2007b) and Souda Bay (Katsanevakis & Thessalou-Legaki, 2009). The main problem is that fan mussels cannot anchor adequately, in a fixed vertical position, in muddy sediment as they can easily sink into the sediment because of the movement of their valves. Furthermore, high silt content may have negative effects on respiration and feeding (Thorson, 1950; Cheung & Shin, 2005). Fan mussels lack siphons but instead have an open pallial cavity, which offers them a fairly high pumping rate, but at the cost of high vulnerability to the entry of sediments (Butler et al., 1993). This explains the absence of *P. nobilis* from muddy areas and, in general, areas of severe sediment disturbance, where only siphonate infaunal bivalves may thrive (Butler et al., 1993).

In Gera Gulf, *Pinna nobilis* reached its highest density in *Posidonia oceanica* meadows. Lower densities were observed in mixed and sandy habitats. This observation concurs with the widely reported fidelity of *P. nobilis* for *P. oceanica* seagrass meadows (e.g. Rabaoui et al., 2010; Vázquez-Luis et al., 2014) and other vegetated habitats, such as beds of the seagrasses *Cymodocea nodosa* and *Halophila stipulacea* or the green alga *Caulerpa cylindracea* (Katsanevakis & Thessalou-Legaki, 2009). Nevertheless, high densities are also found on unvegetated bottoms, especially in areas of low hydrodynamism, such as Lake Vouliagmeni (Katsanevakis 2006). The main factors of a “preference” for seagrass meadows seem to be protection from intense hydrodynamism, good substrate for anchoring, lower mortality caused by predators, and limited poaching by free divers. Seagrass beds dissipate wave energy and attenuate flow (Hendriks et al., 2007), thus reducing the drug experienced by *P. nobilis*, which thrives within seagrass canopies (Hendriks et al., 2011). Hence, seagrass beds have a sheltering effect on fan mussels, as hydrodynamic stress and mortality caused by storms is reduced in comparison to unvegetated bottoms. Furthermore, the robust network of rhizomes in seagrass beds provides firm anchoring points for fan mussels through their byssus threads (Basso et al., 2015). In addition, fan mussels, especially juveniles, are less vulnerable to predation as they are well camouflaged in a seagrass canopy. Similarly, it is more difficult for poachers to spot fan mussels living on seagrass beds than individuals on unvegetated bottoms, where poaching may lower fan mussel populations (Katsanevakis, 2007a).

**Significance of *P. nobilis* population in the Gera Gulf**

The average population density estimated in Gera Gulf is 5.3 individuals per 1000 m², which is very similar to the densities estimated for the other two assessed populations in Greece, namely those of Lake Vouliagmeni (5.7 individuals per 1000 m²) and Souda Bay (8.9 individuals per 1000 m²). However, much higher average densities, by 1 to 2 orders of magnitude, have been recorded in the Mediterranean (Table 3). In Lake Vouliagmeni, there was evidence of very high population densities in the past (see Supplementary file of Katsanevakis, 2016), ~3 to 4 orders of magnitude higher than the current population.
densities, i.e. thousands or tens of thousands of individuals per 1000 m². It has been indicated that poaching is one of the main reasons for the low observed densities in Lake Vouliagmeni (Katsanevakis, 2007a, 2009). Anecdotal information suggests that the level of poaching in Gera Gulf is substantial, and fan mussels are continuously illegally fished and even served in local seafood restaurants. The fact that large individuals were scarce and no individual with width > 19.65 cm was detected, in contrast to Lake Vouliagmeni, where there were many larger individuals (Katsanevakis, 2006), could be due to higher mortality rates or lower growth rates in Gera Gulf (but targeted investigation is needed to reach any solid conclusions).

Nevertheless, the *P. nobilis* population of Gera Gulf is the largest recorded population in Greece, followed

Table 3. Average population densities of *Pinna nobilis* in various Mediterranean sites (modified and updated from Rouanet et al., 2015; Katsanevakis, 2016).

<table>
<thead>
<tr>
<th>Location</th>
<th>Average population density (individuals /1000 m²)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Port-Cros Island (Port-Cros National Park, MPA), Provence, France</td>
<td>10</td>
<td>Vicente et al., 1980; Combelles et al., 1986</td>
</tr>
<tr>
<td>Scandola marine reserve (MPA), Corsica</td>
<td>10</td>
<td>Combelles et al., 1986</td>
</tr>
<tr>
<td>Croatia, Adriatic Sea</td>
<td>90</td>
<td>Zavodnik et al., 1991</td>
</tr>
<tr>
<td>Chafarinas Islands, Spain, Northern Africa</td>
<td>32</td>
<td>Guallart, 2000</td>
</tr>
<tr>
<td>Scandola marine reserve (MPA, NTZ), Corsica</td>
<td>60</td>
<td>Charrier et al. (mentioned in Rouanet et al., 2015)</td>
</tr>
<tr>
<td>Mljet National Park (MPA), Croatia, Adriatic Sea</td>
<td>20-200</td>
<td>Šiletić &amp; Peharda, 2003</td>
</tr>
<tr>
<td>Murcia, Almeria and Balearic Islands, Spain</td>
<td>100</td>
<td>Garcia-March, 2003</td>
</tr>
<tr>
<td>Lake Vouliagmeni, Greece</td>
<td>5.7</td>
<td>Katsanevakis, 2006</td>
</tr>
<tr>
<td>Columbretes marine reserve (MPA), Castellón, Comunitat Valenciana, Spain</td>
<td>15</td>
<td>Garcia-March &amp; Kersting, 2006</td>
</tr>
<tr>
<td>Mar Grande of Taranto, Ionian Sea, Italy</td>
<td>0-0.07</td>
<td>Centoducati et al., 2007</td>
</tr>
<tr>
<td>Soudua Bay, Crete Island, Greece</td>
<td>8.9</td>
<td>Katsanevakis &amp; Thessalou-Legaki, 2009</td>
</tr>
<tr>
<td>Port-Cros Island (Port-Cros National Park, MPA), Provence, France</td>
<td>20-80</td>
<td>Vicente, 2009</td>
</tr>
<tr>
<td>Porquerolles Island, Provence, France</td>
<td>2-23</td>
<td>Vicente, 2009</td>
</tr>
<tr>
<td>Scandola marine reserve (MPA, NTZ), Corsica</td>
<td>140</td>
<td>Vicente, 2010</td>
</tr>
<tr>
<td>Tunisia (east and southeast coast)</td>
<td>15</td>
<td>Rabaoui et al., 2010</td>
</tr>
<tr>
<td>Pass between Bagaud and Port-Cros Islands (Port-Cros National Park, MPA), Provence, France</td>
<td>60-130</td>
<td>Rouanet et al., 2012</td>
</tr>
<tr>
<td>Embiez Island, Six-Fours-les-Plages, Provence, France</td>
<td>19</td>
<td>Trigos et al., 2013</td>
</tr>
<tr>
<td>Cabrera National Park MPA, Majorca Island, Spain</td>
<td>38</td>
<td>Vázquez-Luis et al., 2014</td>
</tr>
<tr>
<td>Javea, Alicante, Spain</td>
<td>&lt;10</td>
<td>Garcia-March, pers. comm. (mentioned in Rouanet et al., 2015)</td>
</tr>
<tr>
<td>Moraira, Alicante, Spain</td>
<td>10-120</td>
<td>Garcia-March, pers. comm. (mentioned in Rouanet et al., 2015)</td>
</tr>
<tr>
<td>West Sardinia, Italia</td>
<td>41</td>
<td>Coppa et al., 2015</td>
</tr>
<tr>
<td>Mar Menor, Spain</td>
<td>22</td>
<td>Belando et al., 2015</td>
</tr>
<tr>
<td>Harbour bay of Favignana island, Italy</td>
<td>110</td>
<td>D’agostaro et al., 2015</td>
</tr>
</tbody>
</table>
by the population of Souda Bay, which was estimated at 139,000 individuals (95% CI: 100,600–170,400). As Gera Gulf is part of the Natura 2000 network of protected areas, contrary to all other known areas in the Aegean Sea with important fan mussel populations, its importance for the conservation of the species is high.

Concluding remarks

The key message from the comparison of the design-based and the model-based approach is that in studies of animal abundance caution is needed when deciding to stratify the study area, especially if the prior information used for stratification is of low quality. In any case, sufficient sampling effort should also be focused on the assumed “low-density” strata, as total abundance there might end up being of the same order of magnitude or higher than in the “high-density” strata.

Wave action and poaching have probably contributed to the size segregation of fan mussels observed in Gera Gulf, with large individuals being less common in the very shallow zone. The limited Posidonia oceanica beds of Gera Gulf largely act as refuge areas for fan mussels, protecting them from poaching, predation and intense hydrodynamism. Further research is needed to assess the level of impact of poaching at population level, as the analogy with other well-studied areas (i.e. Lake Volviagmeni) suggests that population-level impacts of increased fishing mortality are quite probable. Better law enforcement to confront poaching on the species, and additional management actions for the protection of the species (see e.g. Katsanevakis, 2006, 2007a) and its preferred habitats are needed to conserve this important population, which may act as a source for neighbouring areas through larval spill-over.

It is of great importance to continue monitoring the fan mussel population of Gera Gulf in the future. Only with regular monitoring and additional studies will it be possible to detect population trends and understand the dynamics of the species. In particular, in view of the ongoing massive mortality of the species in the western Mediterranean, urgent adaptation of monitoring plans to detect mass mortality events in all Mediterranean fan mussel populations and identify resistant individuals has been suggested (Vázquez-Luis et al., 2017).

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