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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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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 antero-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.

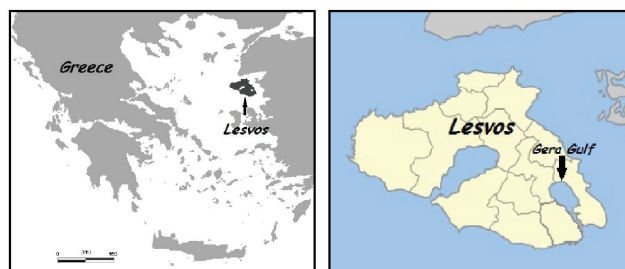
In addition to the general prohibition on its exploitation and marketing, the NATURA 2000 network can contribute to the protection of important populations of *P. nobilis* and its important habitats, such as *Posidonia oceanica* meadows. NATURA 2000 is one of the world's most extensive networks of conservation 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.



Gera Gulf - Study Area



Fig. 1: Gera Gulf and its location in the Aegean Sea. The eighteen sampling stations are indicated.

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-

ture, a subgroup of 13830 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_j) 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_x), the perpendicular distance from the line (L_y) and shell size (S_j), 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

$g(y) = e^{\left(\frac{-y^2}{2\sigma^2}\right)}$, and the two-parameter hazard-rate model

$g(y) = 1 - \exp\left[-\left(\frac{y}{\sigma}\right)^{-b}\right]$, where σ is a scale parameter and b a shape parameter (Buckland *et al.*, 2001). It is possible to include covariates v_j in these models, i.e. variables that may affect detectability, through the scale parameter σ , according to the equation:

$$\sigma = \exp(\beta_0 + \beta_1 v_1 + \beta_2 v_2 + \dots + \beta_j v_j)$$

where β_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,

Table 1. Parametrization of the 16 candidate models m_i for the detection function, average probability of detection P_a (\pm SE), Akaike differences Δ_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.

model	function	covariate	No. of parameters	P_a	Δ_i	population density	abundance	95% CI of abundance
m_1	Half-normal	-	1	0.580 \pm 0.070	15.19	0.0028	112800	64200-198400
m_2	Hazard-rate	-	2	0.740 \pm 0.060	17.98	0.0022	88900	50900-155300
m_3	Half-normal	size	2	0.585 \pm 0.055	16.98	0.0029	112900	64500-197500
m_4	Hazard-rate	size	3	0.725 \pm 0.050	20.34	0.0024	90500	51800-158000
m_5	Half-normal	visibility	2	0.575 \pm 0.055	12.67	0.0029	114300	65300-199900
m_6	Hazard-rate	visibility	3	0.690 \pm 0.055	16.99	0.0024	95400	54600-166600
m_7	Half-normal	habitat	3	0.560 \pm 0.060	2.31	0.0030	118000	67300-206700
m_8	Hazard-rate	habitat	4	0.695 \pm 0.055	6.76	0.0025	94500	54100-165200
m_9	Half-normal	visibility & size	3	0.575 \pm 0.055	14.38	0.0029	114300	65300-200000
m_{10}	Hazard-rate	visibility & size	4	0.690 \pm 0.055	18.89	0.0024	95600	54700-167100
m_{11}	Half-normal	habitat & size	4	0.550 \pm 0.050	2.17	0.0030	119100	68000-208700
m_{12}	Hazard-rate	habitat & size	5	0.700 \pm 0.055	9.34	0.0025	93800	53700-163800
m_{13}	Half-normal	habitat & visibility	4	0.550 \pm 0.050	0.00	0.0030	120100	68500-210400
m_{14}	Hazard-rate	habitat & visibility	5	0.720 \pm 0.060	10.29	0.0025	91500	52400-159800
m_{15}	Half-normal	Habitat & size & visibility	5	0.545 \pm 0.055	0.73	0.0031	120900	69000-211900
m_{16}	Hazard-rate	Habitat & size & visibility	6	0.695 \pm 0.060	6.86	0.0026	94800	54200-165800

whereas the hazard rate function was used in those with an even index. In models m_1 and m_2 , the σ 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 de-

tection 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,



Fig. 2: Map of the study area, which was stratified to apply the design-based method. Stratum 1 corresponds to the 200m buffer zone from the coastline and Stratum 2 to the central part of the gulf.

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 $\bar{D}_h = \frac{\hat{n}_{ct,h}}{A_{ct,h}}$, where $A_{ct,h}$ is the surface of the covered transects in stratum h . The stan-

dard error of the population density at each stratum was estimated as $SE_{\bar{D}_h} = \sqrt{\frac{\sum (D_i - \bar{D}_h)^2}{n(n-1)}}$, where D_i is the estimated density at each transect. The overall population density in the entire study area was estimated as $\bar{D}_{tot} = (A_1 D_1 + A_2 D_2) / A$, where $A = A_1 + A_2$ is the total study area. Total abundance was estimated as $\hat{N}_{tot} = A \bar{D}_{tot}$, and the corresponding standard error was obtained from the equation $SE_{\hat{N}_{tot}} = A \sqrt{W_1^2 SE_{\bar{D}_1}^2 + W_2^2 SE_{\bar{D}_2}^2}$, 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 $\hat{n}_i = \sum_{j=1}^n \frac{1}{\hat{p}_{ij}}$ (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

$$f(E[\hat{n}_i] / A_s) = c + \sum_m s_m(z_{mi}) + \sum_r F_{ri}$$

where f is the link function, c is the intercept, $s_m(\cdot)$ is the 1-dimensional smooth function for the predictor variable m , z_{mi} is the value of predictor variable m for segment i , F_r 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-poisson distribution and logarithmic link were used. The latter ensures positive values for the mean response. The smooth function $s_m(\cdot)$ was represented using cubic regression splines, estimated by penalized iterative least squares (Wood, 2006). Four different GAM models were created; h_1 with no predictor, h_2 with habitat type as predictor, h_3 with depth as predictor, and h_4 with both hab-

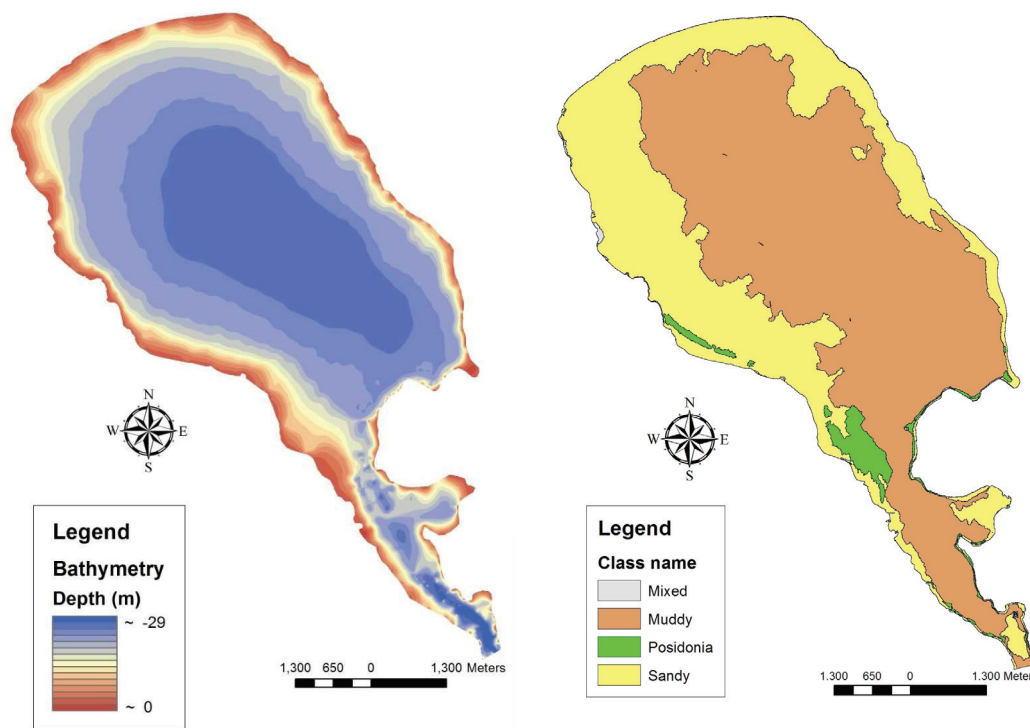


Fig. 3: Bathymetry (left panel) and habitat map (right panel) of Gera Gulf.

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 $E[\hat{n}_r]$ 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. $\hat{N} = \sum E[\hat{n}_r]$. 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

$$[cv(\hat{N})]^2 = [cv(\hat{p})]^2 + [cv(\hat{N}_{DSM})]^2, \text{ where } cv(\hat{p}) \text{ is}$$

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

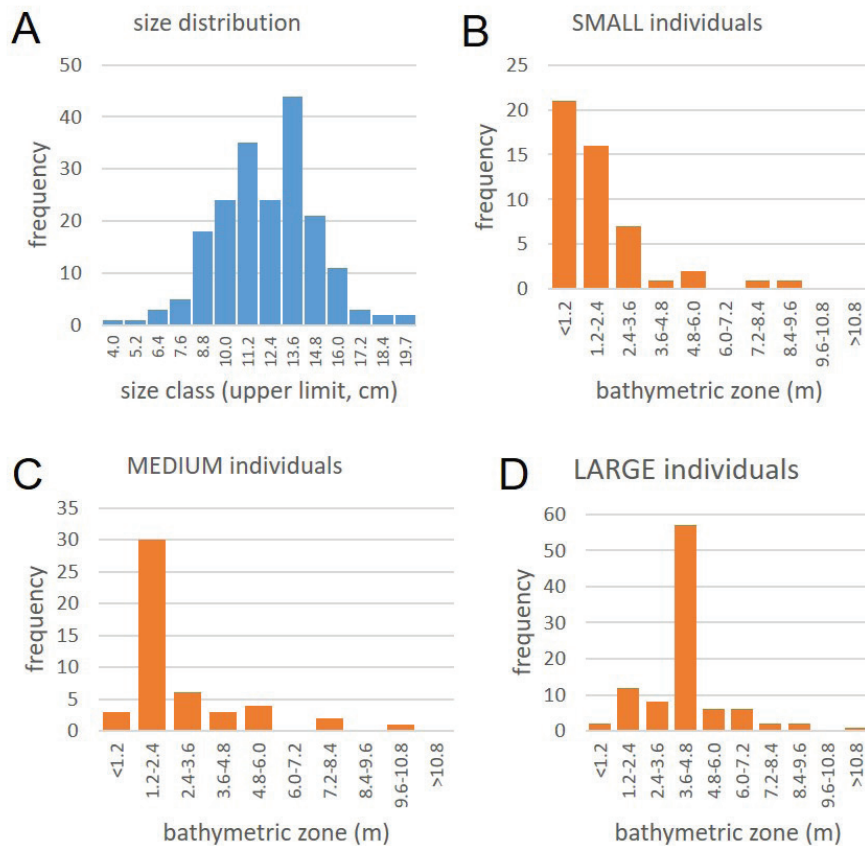


Fig. 4: (A) Size distribution of all recorded *Pinna nobilis* individuals in Gera Gulf; (A–C) Bathymetric distributions of small (shell width <9.9 cm), medium (shell width between 9.9–11.6 cm) and large (shell width >11.6 cm) fan mussels.

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_4 that included both depth and habitat type as predictor variables, was the best model for DSM (Table 2). The expression of h_4 is $f(E[\hat{n}_i]/A_s) = c + s(d) + F(H)$, where $c (\pm SE) = -4.50 (\pm 0.59)$, $A_s = 42 \text{ m}^2$, while the smooth function for depth $s(d)$ and the categorical predictor $F(H)$ are given in Fig. 6. Population density was

Table 2. Evaluation of the 4 candidate GAMs for the population density of *P. nobilis* in the Gera Gulf, based on their generalized cross validation (GCV) score. The percentage of deviance explained by each model and the abundance estimation in the study area are provided.

Model	Spatial Covariate	CV Score	Deviance explained (%)	Abundance estimation
h_1	-	555.5	0%	226600
h_2	habitat type	486.5	18.6%	277400
h_3	depth	507.5	16.7%	175700
h_4	habitat type + depth	460.8	26.2%	213300

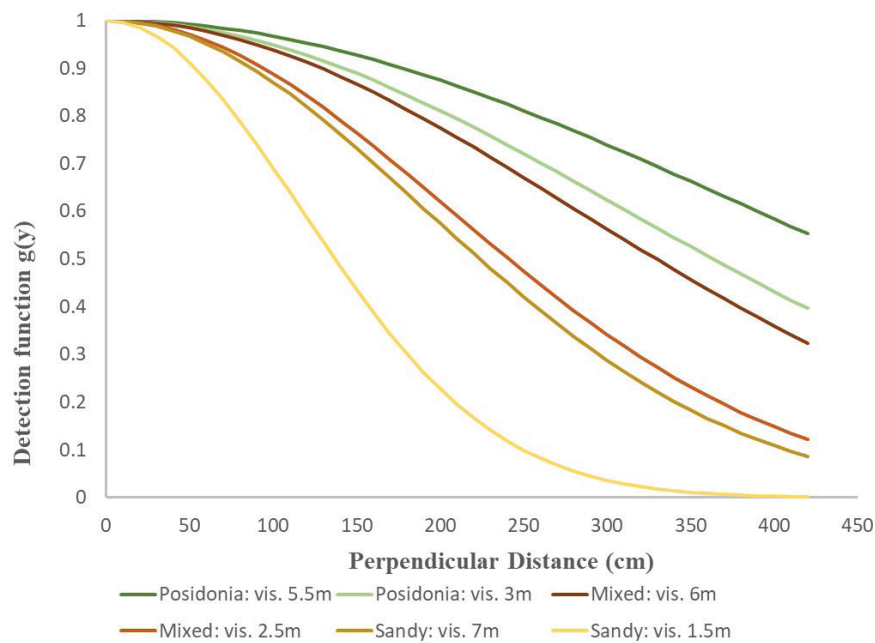


Fig. 5: Best detection function (model m_{13}) of *P. nobilis* for the three habitat types in which individuals were detected (*Posidonia oceanica* meadows, mixed bottoms, and sandy bottoms). For each habitat type, two curves are depicted, corresponding to the minimum and maximum observed visibility at each habitat type.

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

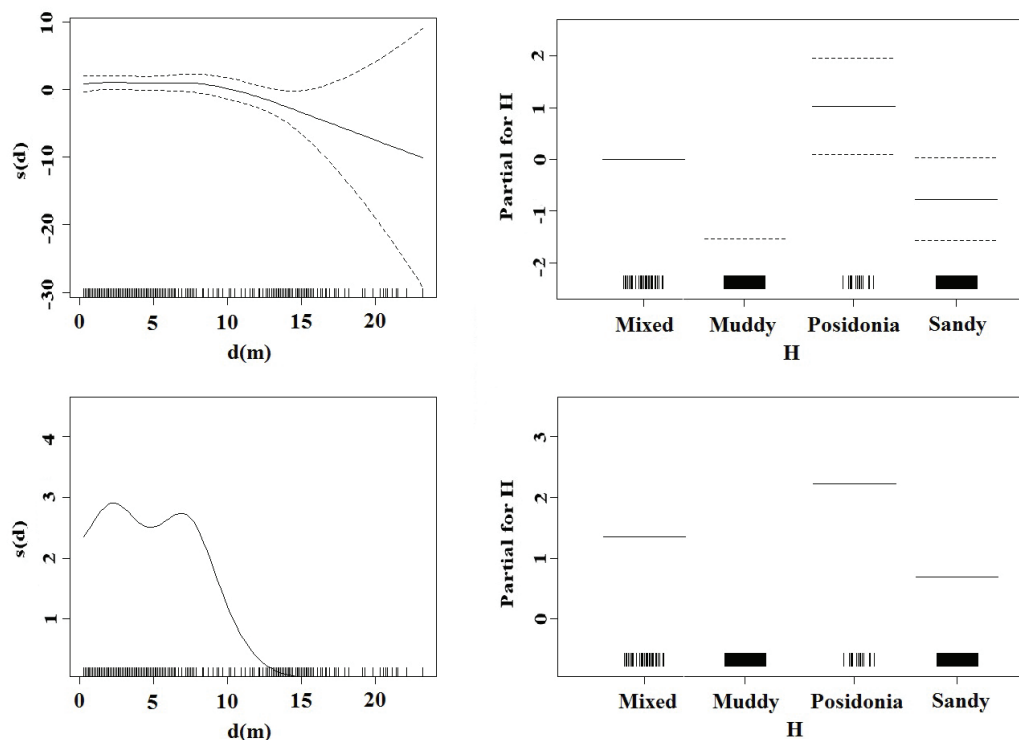


Fig. 6: Estimated smooth term $s(d)$ (depth) and the categorical predictor H (habitat type), for model h_4 of fan mussel abundance in 5x5 m plots in Gera Gulf. In the upper panels, the terms are given in the linear predictor scale and the respective 95% confidence intervals are given with dotted lines. In the lower panels, the terms are given in the response scale (exp-transformed). At the bottom of each graph there is a 1-dimensional scatter plot illustrating the distribution of available data.

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-Luis *et al.*, 2014) and although density declined with depth,

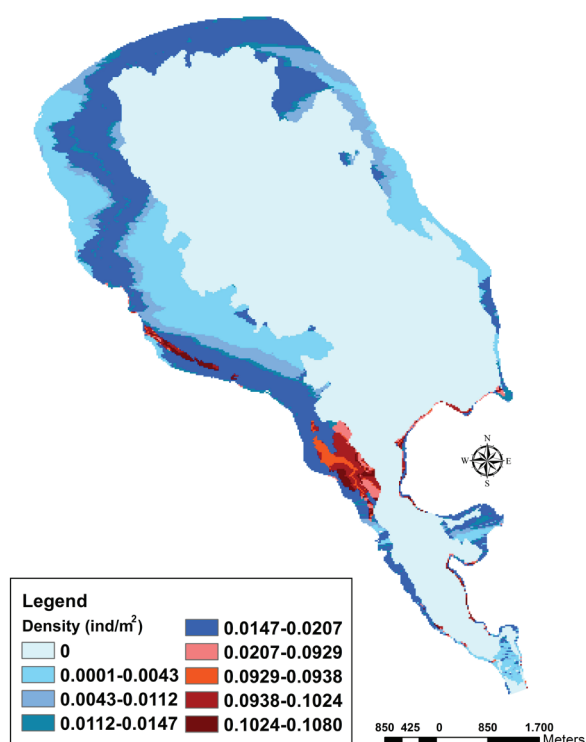


Fig. 7: *Pinna nobilis* population density map based on the model-based approach and on density model h_r .

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.*, 2007) 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 drag 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

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

Location	Average population density (individuals /1000 m ²)	Source
Port-Cros Island (Port-Cros National Park, MPA), Provence, France	10	Vicente <i>et al.</i> , 1980; Combelles <i>et al.</i> , 1986
Scandola marine reserve (MPA), Corsica	10	Combelles <i>et al.</i> , 1986
Croatia, Adriatic Sea	90	Zavodnik <i>et al.</i> , 1991
Chafarinas Islands, Spain, Northern Africa	32	Guallart, 2000
Scandola marine reserve (MPA, NTZ), Corsica	60	Charrier <i>et al.</i> (mentioned in Rouanet <i>et al.</i> , 2015)
Mljet National Park (MPA), Croatia, Adriatic Sea	20-200	Šiletić&Peharda, 2003
Murcia, Almeria and Balearic Islands, Spain	100	García-March, 2003
Lake Vouliagmeni, Greece	5.7	Katsanevakis, 2006
Columbretes marine reserve (MPA), Castellón, Comunitatvalenciana, Spain	15	García-March & Kersting, 2006
Mar Grande of Taranto, Ionian Sea, Italy	0-0.07	Centoducati <i>et al.</i> , 2007
Souda Bay, Crete Island, Greece	8.9	Katsanevakis & Thessalou-Legaki, 2009
Port-Cros Island (Port-Cros National Park, MPA), Provence, France	20-80	Vicente, 2009
Porquerolles Island, Provence, France	2-23	Vicente, 2009
Scandola marine reserve (MPA, NTZ), Corsica	140	Vicente, 2010
Tunisia (east and southeast coast)	15	Rabaoui <i>et al.</i> , 2010
Pass between Bagaud and Port-Cros Islands (Port-Cros National Park, MPA), Provence, France	60-130	Rouanet <i>et al.</i> , 2012
Embiez Island, Six-Fours-les-Plages, Provence, France	19	Trigos <i>et al.</i> , 2013
Cabrera National Park MPA, Majorca Island, Spain	38	Vázquez-Luis <i>et al.</i> , 2014
Javea, Alicante, Spain	<10	García-March, pers. comm. (mentioned in Rouanet <i>et al.</i> , 2015)
Moraira, Alicante, Spain	10-120	García-March, pers. comm. (mentioned in Rouanet <i>et al.</i> , 2015)
West Sardinia, Italia	41	Coppa <i>et al.</i> , 2015
Mar Menor, Spain	22	Belando <i>et al.</i> , 2015
Harbour bay of Favignana island, Italy	110	D'agostaro <i>et al.</i> , 2015

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

by the population of Souda Bay, which was estimated at 139000 individuals (95% CI: 100600–170400). 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 Vouliagmeni) 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 larvae 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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