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The population of *Pinna nobilis* in the Mar Menor coastal lagoon: A story of colonization and uncertainty

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Running page head: Colonization of *Pinna nobilis* in the coastal lagoon of Mar Menor

Abstract: The Mediterranean fan mussel (*Pinna nobilis*) populations have progressively fallen during the last decades as a result of economic activities. But the threat has become particularly severe since 2016, concurrently with a mass mortality event triggered by the parasite *Haplosporidium pinnae* and it exist evidences that *Mycobacterium* species could have a major role in the event. Indeed, the epidemic has spread throughout the Mediterranean, although coastal lagoons seem to offer a degree of "resistance" against the parasite. By the early 1980s *P. nobilis* appeared in the Mar Menor lagoon and rapidly became an important component of the benthos. However, the fan mussel colonizing process in the lagoon was cut short in 2016 when a massive mortality occurred, possibly as a consequence of the environmental collapse that occurred in the lagoon, in parallel to the mortality that the species suffered in the Mediterranean that same year. In this contribution, the spatial distribution of *P. nobilis* in the Mar Menor is estimated for three time-spans: a) 2003-2004, b) 2013, and c) 2016. The first two time-spans using published data, and the last one using data collected in a new campaign. The probability of occurrence for the three time-spans was estimated using Random Forest and Random Forest Regression-Kriging

models. The main environmental variables that determined the dispersion and colonization of the bivalve in the lagoon before 2016 are also identified.

Key words: Coastal lagoon; *Pinna nobilis*; hypersalinity; SDMs models; Machine learning; Random Forest

1. INTRODUCTION

The "fan mussel" (*Pinna nobilis* Linnaeus, 1758) is a Mediterranean endemic filter-feeding bivalve, the largest Mediterranean bivalve and one of the largest in the world, attaining lengths up to 120 cm (Zavodnik et al. 1991). According to Butler et al. (1993), it colonizes coastal areas with salinity ranging from 35 psu to 42 psu, at depths between 0.5 and 60 m, and mostly in soft-sediment areas overgrown by meadows of the seagrasses *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera marina* and *Zostera noltei* (Zavodnik 1967, Zavodnik et al. 1991) or sandy bottoms (Katsanevakis 2007). The population of *P. nobilis* in the Mediterranean Sea had been greatly reduced during the last few decades as a result of recreational and commercial fishing, collection by divers and incidental killing by anchoring (Rabaoui et al. 2011). But the real crisis came when from early autumn 2016 a mass mortality event (MME) was detected, impacting *P. nobilis* throughout the western Mediterranean Sea due to a parasite *Haplosporidium pinnae* (Darriba 2017, Catanese et al. 2018, Cabanellas-Reboredo et al. 2019). Carella et al. (2019) also found evidences that a *Mycopacterium* species could have a major role in the MME. Specimens of all sizes, depth ranges and habitat types were affected, and the mortality has reached 100% in most populations (Vázquez-Luis et al. 2017). The rapid collapse of the population caused concern and a change of status from the "Vulnerable" category to "Critically endangered" with a serious risk of extinction (Orden TEC / 1078/2018). Since then, the epidemic has spread through much of the Mediterranean; however, coastal lagoons seem to be places where the mortality has not spread (Tsatiris et al. 2018, Panarese et al. 2019).

Coastal lagoons are transitional and dynamic zones separated from the ocean by geographical and ecological barriers; they are shallow inland water bodies connected with the sea by one or more restricted inlets which remain open at least intermittently (Kjerfve 1994). Coastal lagoons are controlled by complex interactions between land, ocean and atmosphere. The ecotone character of coastal lagoons makes them diverse, dynamic and fragile systems (Giménez-Casalduero 2006, Viaroli et al. 2007). However, human activities cause changes in the processes and ecological interactions of these ecosystems (Basset et al. 2008), and the increasingly intensive agricultural activity that takes place in the land of the surrounding areas increases nutrient inputs and can even provoke severe episodes of eutrophication (McGlathery et al. 2007).

The Mar Menor is a hypersaline coastal lagoon, located in a semi-arid region of south-eastern Spain. Its insulation has caused extreme environmental conditions, such as temperature stress (with abrupt temperature changes even daily) and high salinity, which have allowed the presence of communities dominated by euryhaline and eurythermal species that generated a characteristic landscape (Mas et al. 2017). In 1976, one of the inlets (Estacio) was dredged and widened, making it 30 m wide and 5 m deep (Mas 1994). This connection modified the hydrodynamic regime of the lagoon, altering the water renewal rate. As a consequence, its sedimentary nature changed, salinity decreased, and annual and daily temperature changes were damped, approaching values similar to those of the Mediterranean Sea (Baraza et al. 2003, Lloret et al. 2005). As a result, ecological barriers were weakened, allowing the entry and establishment of new species in the lagoon, one of them was *P. nobilis*. The species that have colonized the lagoon since the widening of the inlet vary from the typically Mediterranean *P. nobilis* to species considered invasive such as the bivalve *Bursatella leachii* De Blainville, 1817 (Zenetos et al. 2004), or the blue crab (*Callinectes sapidus* Rathbun, 1896) (Gimenez-Casalduero et al. 2016).

Although *P. nobilis* is a common bivalve in semi-closed bays and Mediterranean coastal lagoons (Katsanevakis 2004), it was absent from the Mar Menor until the early 1980s, when was observed by Rodríguez Babio & Navarro Tárrega (1983) and Murillo & Talavera (1983), and in a few years became an important component of the benthos (Belando et al. 2014). To model their potential habitat up to 2015, it is important to know the routes of entry into the lagoon and the environmental factors that determined its spread and colonization patterns in the lagoon. After this wide stabilization process, in 2016 a high mortality event occurred in the Mar Menor. All the results indicate that the cause of this mortality is not due to the *Haplosporidium pinnae* epidemic (Catanese et al. 2018), but the environmental collapse that occurred in the lagoon in 2016.

This environmental collapse started in the second half of 2015, when, after decades of nutrient and phytosanitary inputs from nearby agricultural activities, the Mar Menor coastal lagoon underwent a Harmful Algal Bloom (HABs) event. In spring 2016, the situation had reached a dramatic peak, the high concentration of phytoplankton prevented the light from reaching the deeper areas of the lagoon, limiting photosynthesis in these areas (CACMM 2017). The vegetation below the new photic threshold died and the organic matter which accumulated on the bottom caused processes of hypoxia and anoxia. The absence of oxygen caused the death of the entire benthic community below 3 m depth and more than 85% of the lagoon surface was affected (Belando et al. 2017).

We have estimated the potential distribution and density of *P. nobilis* using species distribution models (SDMs). The advantage of this type of methods is that environmental/geographic information is used to explain observed patterns of species presence or abundance and density (Elith & Graham 2009). In this study a machine learning approach was used, which is a more robust alternative to parametric methods. The complex nature of ecological systems hinders our ability to generate accurate models using the traditional frequentist data model. Problems such as complex non-linear interactions, spatial and temporal autocorrelation, high dimensionality, homocedasticity or multivariate normality are difficult to address by these methods, whereas they are more easily dealt with by machine learning algorithms (Evans et al. 2011). Although the application of SDMs to the marine environment has not been widespread, recent technological advances in the collection and analysis of data has increasingly facilitated their use in wildlife habitat evaluation. Reiss et al. (2015) highlighted the importance of such methods in marine benthic ecosystems, and extensively reviewed the most used and their applications.

The main objectives of this study were (1) to characterise the *P. nobilis* population in the lagoon before the environmental collapse of 2016, (2) identify the main entry routes into the lagoon and analyse the colonization process before 2016, (3) identify the main factors that determine the distribution of the species in the lagoon and (4) carry out a first approximation of the situation of the *P. nobilis* lagoon population after the eutrophication crisis.

2. MATERIALS AND METHODS

2.1. Study site

The Mar Menor (Fig. 1), located in the south-western Mediterranean Sea (37º42'00''N 00º47'00''W), is the largest coastal lagoon in the western Mediterranean. It covers an area of 135 km^2 with a mean depth of 3.5 m and a maximum of 7 m. The lagoon is the lowest area of the Campo de Cartagena basin. This basin has an extension of 12,500 ha and there are water inputs by 13 ephemeral channels that flow into the lagoon. The Rambla del Albujón (66,700 ha) is the most important watercourse both in extension and in sediment and nutrient flows (Fig. 1B). It is a strongly anthropized area, mainly due to the expansion of intensive irrigation agriculture and tourism in the last few decades. Such activities are located very close to the lagoon, generating many environmental problems, because of their intensification that has resulted in a modification of water and nutrient flows (Esteve-Selma et al. 2016).

The area is characterized by low precipitation (<300 mm yr^{-1}), high insolation and irradiation, and high evaporation rates. Because of these factors, it is a hypersaline (38-47 psu) with warmer waters in summer and colder in winter relative to the adjacent Mediterranean Sea (10-32 ºC). Connection of the lagoon with the Mediterranean Sea was limited due to the presence of a sand bar called La Manga, crossed by three very shallow channels (Lillo 1979, López-Bermúdez et al. 1981). But the Estacio canal widening increased the connection between the lagoon and the Mediterranean and caused a decrease in salinity, which meant the elimination of one of the main environmental barriers for the survival of most species. The drop-in salinity favoured the entry and colonization of new species from the Mediterranean. The surveys carried out by Murillo & Talavera (1981) for the Mar Menor malacological study, did not detect the presence of *P. nobilis* in the lagoon. It was in 1982 when the first specimens were identified (Rodríguez Babio & Navarro Tarrega 1983).

2.2. *Pinna nobilis* data

In this study, several sources of information were used to analyse the colonization and dispersal processes of *Pinna nobilis* in the lagoon, (Fig. 2): (A) Historical data, obtained from published data (Rodríguez Babio & Navarro Tarrega 1983) and Murcia Region Sites of Community Importance (SCI) database (CARM 2003); (B) Integrated 2013-14 data-set and complementary 2016 data-set (to estimate the surviving specimens for that year), obtained from specific sampling surveys carried out for the present study (2013 and 2016), and published data (Belando et al. 2014). Table 1 summarizes the data-sets used in this study.

2.2.1. Historical data

The location where the first *P. nobilis* specimens were recorded in 1983 was included (Fig. 2A) (Rodríguez Babio & Navarro Tárrega 1983). In order to characterize the species distribution in 2003-04, the Murcia Region marine LIC database was used (Fig. 2A). This database includes 1646 presence/absence observation points sampled during 2003 and 2004; however, it is a very unbalanced data-set with 34 presence observations and 1611 absence points due to the kind of sampling that was carried out aimed at monitoring the lagoon habitats. Data comes from the database of marine SCIs of the autonomous community of the region of Murcia. Scuba diving and snorkel transects were made for mapping the biocenoses and key species such as seahorse, sea needle or fan mussel presence were recorded (CARM 2003). Some biocenoses (such as sandy areas near the shore), with a very scarce presence of *P. nobilis*, were oversampled.

Using an imbalanced data-set to train a classification algorithm may produce a bias towards the most frequent class, negatively impacting the model's performance (Krawczyk 2016). A high specificity but a low degree of sensitivity can be obtained, which affects the results when calculating presence probability. Kuhn & Johnson (2013) describe several strategies to solve such problems when modelling, and they recommend a cluster-based selection strategy to specify points with similar environmental conditions based on the input covariates. In order to implement this strategy, a distance-based filtering was, firstly, performed to eliminate observations with a distance of less than 20 from each other (in the data set there are 674 observations that have at least one second point at this distance). Second, a stratified down-sampling of the absence data was performed. The strata (5 areas) were obtained by a hierarchical cluster method based on Strauss & von Maltitz (2017): Manhattan distance for dissimilarity, Wald's method for aggregation and the same variables used to fit the model (Fig. 2A). Inside each stratum a number of points proportional to the total of points in it were randomly removed. As a result, a new data-set was obtained with 84 points (35 presences and 49 absences, Fig. 2A).

2.2.2. Integrated 2013-14 data-set and 2016 data-set

Six surveys were conducted over a period of eight months, between March and October 2013 (Fig. 2B). The lagoon was divided into a 49 square grid (Fig. 2B) with a base resolution of 2000 m to obtain maximum spatial coverage to characterize the presence and density distribution of the bivalve. A total of 60 density measure fan mussel density were sampled. Specifically, 29 stations of SCUBA diving took place and three line transects of 30 m long and 2 m width were carried out in each station. All individuals within the transect were counted.

Additionally, 25 density measurements were conducted by bathyscaphe. Thus a 200 long visual sampling area of two m width was surveyed. All specimens within the transects area were counted.

This sampling was complemented with two line transects using aquaplane by SCUBA diving covering the first one 2007 m and the second 2633 m long and both 2 m wide in the deepest areas (Buckland et al 2001). The diver with the aquaplane was dragged at a speed of approximately 0.5 m / s and the working depth was 1 to 1.5 m above the bottom. As a safety measure, the diver was dragging a small buoy. The track line was geo-referenced from the boat using a GPS (Garmin e-Trex 20) and every minute during the line depth was measured using a portable depth sounder (Hondex PS7). The start and end time of the line was recorded and synchronized between the diver and the boat. The diver recorded the number of specimens found along the route and the minute of the observation. The first track was divided into 11 transects and the second one into 14 transects at a distance of approximately 175 m. The average density of 3 randomly selected transects was estimated at the beginning, middle and end of the track. So 3 density data were obtained for each aquaplane line. The position of each transect was calculated by crossing the time recorded by the sampler with the vessel's track data.

A total of 57 sampling stations were incorporated from the Belando et al (2014) results. According to the authors the sampling was carried out by scuba diving. Three transects of 200 m long and 2 m width were made (Belando et al. 2014).

The data-set from the 2013 field sampling and the 2014 data-set were merged and used to fit a presence-absence model in the lagoon. After checking the quality of the points, 117 were selected with 65 absences and 52 presences (Fig. 2D).

Additionally, after the environmental collapse of 2016 caused by an eutrophication process, a survey was carried out to estimate the surviving specimens. A total of 17 scuba diving station were sampled with the same methodology described above. Three transects (30x2) were carried out in each station. All individuals within the transect were counted. All density data were standardized to number of Pinna nobilis per 100 m^2.

2.3. Environmental data

Eleven spatially distributed environmental variables were considered for the regression models. In the case of the variables obtained from data points (sediment granulometry, organic matter content, salinity and depth), interpolation surfaces were obtained using ordinary kriging (OK) and directional semivariograms or an omnidirectional semivariogram if there were not significant differences among the directional semivariogram. We used the R package *automap* (Hiemstra et al. 2009), which allows the automatization of a weighted least squares optimal estimation of semivariogram parameters using the Gauss-Newton algorithm.

To characterize the sediment during the 2013 campaign, two cores (5 cm diameter and 20 cm length) in the sampling area per square were collected. The granulometric analysis was performed manually in a sieve column according to the Wentworth granulometric scale, and the organic matter of the sediment was determined using the Walkley and Black method (Buchanan 1984). The physicochemical variables of the water column (temperature and salinity) were taken at the epicentre of each square using a CTD sensor simultaneously with density campaigns.

The variables with their sources and processing methods are described below: i) Depth (DEPTH), obtained from bathymetric curves and depth points available from the eco-cartographic studies from the *Dirección General de Sostenibilidad de la Costa y el Mar* (EcoMag 2009); ii) Distance to the shore (DSH); iii) to v) Distance to the channels connecting the lagoon and the Mediterranean Sea: Estacio channel (DEST), Encañizadas channel (DENC) and Marchamalo channel (DMAR) (Fig. 1C); vi) Distance to the Rambla del Albujón outlet (DALB); vii) percentage of silt (SILT, diameter < 0.063 mm), viii) percentage of sand (SAND, 1 ≤ diameter ≥ 0.063 mm.) and ix) percentage of gravel (GRAV, diameter > 1 mm.); x) organic matter (MO); xi) salinity (SAL) interpolated with OK from data compiled previously (González-Wangüemert et al. 2006, González-Wangüemert et al. 2009). In addition, the X and Y coordinates (XCOOR and YCOOR) were also used as predictors as a simple alternative to indicate the trend of geographic space (Evans et al. 2011), proximity and geographic connection between observations (Hengl et al. 2018).

2.4. Modelling framework

2.4.1. Classification and Regression models

Random Forest (RF) (Breiman 2001) was used to obtain the probability of the presence of *P. nobilis* and to determine the potential presence area. This model was selected out of four classification methods that were previously tested to identify the most accurate (Gomariz-Castillo, Giménez-Casalduero & Alonso-Sarría unpub. data): Generalized Lineal Models (GLM), Support Vector Machines (SVM), Random Forest (RF) and Regression-Kriging (RK). These methods for variable selection are implemented in the R package *caret* (Kuhn 2015) and are fully described in Hastie et al. (2009) and James et al. (2013). As proposed by Lauria et al. (2017), the abundance (density of individuals) was predicted only in the areas of high probability of presence that were previously estimated using the same methods.

RF is a non-parametric algorithm that builds an ensemble of decision trees. Each tree is calibrated using a subsample of cases obtained by bootstrapping. In addition, the features to perform each split are selected from a random subsample of the whole feature set. When all trees have been trained, but not pruned, each new case is analysed by all the trees and the final prediction is obtained as the most frequent result (classification) or by averaging the results (regression). RF uses two parameters: number of trees (n_{tree}), we used the default value (500) since the algorithm is not very sensitive to this parameter (Liaw & Wiener 2002), and the number of features randomly sampled in each split (m_{try}). This last parameter was calibrated to maximise the area under the receiver operating characteristic curve (AUC ROC) in the classification and to minimise the root-mean-square error

(RMSE) in the regression. It also provides a measure of the importance of the variables reflecting the increase in accuracy that they produce in the model.

For the 2013-14 data-set, we used Random Forest Regression-Kriging (RFRK) that includes both a global and a local component. Regression-Kriging (RK) (Hengl et al. 2004) is a spatial prediction technique that combines a regression of the dependent variable on auxiliary variables (global component) with interpolation (OK interpolation) of the regression residuals (local component).

Models were calibrated using a K-Block Cross-Validation (K-Block-CV) strategy. This approach attempts to prevent accuracy overestimation due to spatial dependence between training and validation data. Blocks and folds in k-fold-CV are similar; the fundamental difference is that the latter are randomly sampled and the former correspond to spatial subsets. The size of such blocks is defined after an autocorrelation analysis of the study data. This methodology was proposed by Valavi et al. (2019), and is implemented in the R *blockCV* package. The study of the semivariograms revealed that the distance above which it can be considered that there is no spatial autocorrelation is 6,000 m, for this reason, K=6 blocks, distributed in nine spatial cells, were generated.

To validate the presence/absence models, the area under the ROC function (AUC) (Fielding & Bell 1997), overall accuracy (Acc) and kappa (Cohen 1960) were used. The threshold probability value to decide whether the species was or was not present was calculated using the Youden (1950) method that tries to maximise both specificity and sensitivity. RMSE was used to validate the abundance models. AUC and RMSE were estimated using confidence intervals and the results were used to compare and select the significantly best model.

2.4.2. Variable selection

Although RF is robust in the face of non-informative or redundant predictors, such features can increase uncertainty, reduce the overall effectiveness of the model (Kuhn & Johnson 2013), increase the difficulty of interpretation and negatively impact parameter estimation. There are two basic approaches to feature selection: filtering and wrapping.

Filtering is an approach prior to modelling, in which an importance measure is calculated and the features that do not reach an appropriate value are removed. In this case we used filtering to avoid multicollinearity. In this study, multicollinearity was analysed by estimating a global correlation index obtained by averaging the absolute values of the columns in the correlation matrix (Kuhn & Johnson 2013).

Wrapping methods remove features while calibrating the model in order to select the feature set that maximizes model accuracy. Most wrapping methods add or remove features one by one. In Recursive Feature Elimination (RFE) (Guyon et al. 2002) features are removed in subsets for efficiency reasons. A model is calibrated and the importance of the features used for its calibration is estimated using RF. All features whose importance is below a given threshold are then removed and the process begins once again until all features in the model are considered important enough. RFE was applied using the previously described cross validation scheme. In RFE, the model is calibrated using all features, then the model goodness of fit (using AUC for classification and RMSE for regression) and feature importance were calculated; the model is then recalculated with just the most important features; these process is repeated recursively until the minimum number of variables that maximize the goodness of fit is obtained.

The methodology described was implemented with R software (R Core Team 2018), an Open Source data analysis program, which allows reproducibility to other study areas and data-sets.

Using a filtering approach to eliminate multicollinearity, nine out of the 13 initial covariates were selected (Fig. 3). DMAR, SAND, XCOOR and YCOOR) with VIF values higher than 0.9 were removed from the model. Of note is the strong negative correlation between DMAR and SAL (Fig. 3B) ($p < 0.05$, $r = - 0.8$) and positive correlation between DMAR and MO (Fig. 3G) ($p < 0.05$, $r = 0.92$), and between DMAR and YCOOR ($p < 0.05$, $r = 0.93$); SAND is highly correlated with SILT (Fig. 3H) ($p < 0.05$, $r = -0.98$) and DALB ($p < 0.05$, $r = -0.84$). Fig. 3 shows the interpolated surface of the 8 included covariates.

3. RESULTS

3.1. Global evaluation of the models

The model selected to generate the estimated distribution surfaces was RF, with the reduced variable set (after VIF and RFE), for presence/absence data-sets and RFRK for 2013-14 density data-set. In the presence-absence models, very high accuracy was reached for the calibration (accuracy = 100% and AUC=0.99); the values of K-fold-CV were high, with AUC = 0.845 ± 0.033 in the 2003-04 data-set and AUC = 0.85 ± 0.025 in the 2013-14 data-set. In the case of the density model, values of RMSE = 1.755 and r^2 = 0.798 were obtained in calibration; however, the accuracy in K-fold-CV was reduced to r^2 = 0.162 ± 0.015 and RMSE = 2.574. In any case, this RMSE value is still relatively low.

3.2. Variable selection and effects of predictors in selected models

In all three cases, six independent variables were selected to explain the spatial distribution of *Pinna nobilis*. The most important variables (most relevant factors) to explain the pattern of distribution were in general the distances to the connections with the Mediterranean (specially DENC and to a lesser extent DEST) and depth (DEPTH).; in the 2013-14 data-set, gravel percentage (GRAV) was the most important variable in the distribution of *P. nobilis*.

Fig. 4 shows the effects of the predictors on the presence probability of *P. nobilis* for the 2003-04 data-set, from the most to the least important predictor. The four most relevant are DENC (Fig. 4A), DEPTH (Fig. 4B) MO (Fig. 4C) and DEST (Fig. 4D), that is, the distance to the entry points of colonisation, depth and organic matter. For the distances to points connecting the Mar Menor and the Mediterranean (DENC, Fig. 4A, and DEST, Fig. 4D), a somewhat stable value is observed with occurrence probabilities of around 0.5 up to a distance of 10,000 m for DENC and 6,000 m for DEST; for larger distances the probability falls off. In the case of depth, a more or less linear increase in the probability of occurrence is observed from the deepest points until a depth of 3.75 m, where maximum probability is reached, after which an inflection point is observed. This curve behaviour seems to be related to a preference for shallow areas masked by anthropic coastal pressure. In the case of organic matter concentration in the sediment (MO, Fig. 4C), there is an increase in the probability of encountering the bivalve when the concentration of MO reaches values higher than 5%. As far as the distance to the coast (DSH, Fig. 4E) is concerned, a maximum preference is observed at a distance of approximately 500 m, a result consistent with that found for the optimum depth. Regarding salinity (SAL, Fig. 4F), the range of salinity in the period 2003-2004 was between 44 - 45 psu and probability of presence of fan mussels decreased with increasing salinity (González-Wangüemert et al. 2009).

In the period 2013-2014 (Fig. 5) gravel percentage (GRAV, Fig. 5A) was the most important variable in the distribution of *P. nobilis*. The variables that best explain the distribution of the species in the lagoon based on its presence are: i) the percentage of gravel in the sediment (GRAV, Fig. 5A) with a high probability of occurrence beginning above 10%, ii) the distance to the coast (DSH, Fig. 5B), whose optimum seems to have moved away in relation to the data of the previous period, with the highest probability starting at 1,000 m to the coastline, and the foreseeable influence of the distance from the Encañizadas (DENC, Fig. 5C), while there is a decrease in probability as we move away from this point of connection with the Mediterranean. The same result is observed in relation to the distance to the Estacio channel (DEST, Fig. 5E), which creates a buffer of high probability of encounter in the first 5,000 m from the point of connection. In relation with depth (DEPTH, Fig. 5F), probability was higher from 3 m depth. The pattern in relation to salinity (SAL, Fig. 5D) shows a higher probability of occurrence in areas of higher salinity, within the range that characterizes the lagoon although the differences are subtle.

The specimen density results (Fig. 6) show similar patterns to those found with the presence-absence model in terms of distance to the channels (DENC, Fig. 6A) and to Estacio (DEST, Fig. 6F) or distance to the coastline (DSH, Fig. 6E), parameters such as salinity (SAL, Fig. 6B) have an opposite behaviour, there is a *P. nobilis* high density with minimum salinity values within the range described in the lagoon, and there is a peak of bivalve density associated with silt percentage around 40% and organic matter (MO, Fig. 6D) of 4% in sediment.

3.3. Spatial prediction

Figs. 7A and 7B represent the potential distribution of *P. nobilis* according to the predicted probability of occurrence and the potential zone, obtained from the reclassification of the probability using Youden (1950) method as cut-off (0.501 in 2003-04 and 0.503 in 2013-14). It should be noted that, although one of the advantages of RF is that it predicts the presence or absence of the species at all the observed points, the reliability of the resulting surface will depend on the quality of the sampled data; therefore, the distribution in 2003-04 (Fig. 7A), which was obtained in a sampling carried out with an aim other than the observation of *P. nobilis*, may be less reliable. However, the results obtained in 2003-04 after the resampling process and its modelling allow a better understanding of the potential distribution of *P. nobilis* with respect to the partial studies existing in this period.

Comparing the potential area in 2003-04 (Fig. 7A, 2,985 ha, 22% of the Mar Menor coastal lagoon) with that for 2013-14 (Fig. 7B, 7,385 ha, 54.7% of the Mar Menor coastal lagoon), a concentration in the northern part of the lagoon, limited by the central islands, appears in the first sampling, the only exception being a point where the presence of the bivalve is evident next to the entrance of La Gola channel connection.

This spatial pattern could be explained by the high probability of the bivalve being present at the entrances to the lagoon (main routes of entry) and their gradual expansion towards the other areas. In 2013-14 (Fig. 8), although the southward expansion is evident, the highest probability is still observed in the areas closest to the entrances (main routes of entry), which is consistent with the effects shown in Fig 6. A secondary maximum appears to the north of the islands. There is also a southward tendency to spread from the entrances to the islands, and a gradual expansion from there, again towards the south, which may be related to the colonization pattern. The density model estimates values that vary between 0.01-21.6 ind/100m^2, and an average density of 2.181±0.004 ind/100m^2 (excluding density values outside the potential distribution area in 2013-14), so the total estimated number in the lagoon for 2013-14 is 1,609,943±3,309 individuals.

To complete this study, a preliminary sampling was carried out after the eutrophic collapse of 2016 (Fig. 9). Thirteen of the samples correspond to stations with presence of *P. nobilis* (squares in Fig. 9) and four stations with absence (triangles in Fig. 9). A 100% mortality was observed in the 17, 35% stations deeper than 3 m, that is the threshold of the "severe eutrophication" area (Fig. 9). In shallow sampling stations, 60 *P. nobilis* individuals were counted; only 5 (8.79%) of the found specimens were alive.

4. DISCUSSION AND CONCLUSIONS

The population of *Pinna nobilis* has been greatly reduced in most Mediterranean areas in the past few decades due to the human pressure (García-March et al. 2007, Katsanevakis

2007, Katsanevakis & Thessalou-Legaki 2009, Rabaoui et al. 2011). However, from 1983, first observation in the lagoon (Rodríguez Babio & Navarro Tárrega 1983), to 2014, the Mar Menor has witnessed colonization and stabilization of the ”fan mussel”, with a total of 7,385 ha of potential habitat In order to understand this process of *P. nobilis* colonization in the Mar Menor, it is necessary to interpret the dispersion model and the influence of the main factors that determine the colonization process.

Some of the most common mechanisms for species transfer in near shore water include the movement of alien communities on ship bottoms or the connection of waterways through artificial channels (Ruiz et al. 1997). The establishment of foreign species lead the major changes in the composition of the fauna of many closed and semi closed areas such as the estuaries, harbours and coastal lagoons of the western Mediterranean (Cognetti & Maltagliati 2000). This invasion could occur after years to decades of dispersal opportunities from source to recipient region. The successful establishment of alien species often requires many inoculations, and success will depend partly upon inoculant size, the physiological condition of individuals, and local conditions at the time of arrival (Roughgarden 1986). Clearly, the artificial expansion of the Estacio channel has caused the entrance of numerous species into the lagoon. The drop in salinity due to the greater exchange of water with the Mediterranean has generated more favourable conditions for the survival of these species (Gimenez-Casalduero et al. 2016). In the case of *P. nobilis*, the channels seem to have played an important role in the entry of larvae from the nearby Mediterranean populations. The results of this study show that not only the artificial channel of El Estacio, but the natural channel "Encañizadas" has also been an important gateway to the lagoon.

The salinity tolerance range for *P. nobilis* has been described as between 35 and 42 psu (Schlieper et al. 1960, Butler et al. 1993). It is reasonable to think that this species could not support the high salinity levels (average values of 52 psu) in the lagoon before opening the Estacio channel (Aravio-Torre & Arévalo 1971). But the presence of the “fan mussel” since the early 1980s, few years after opening the communication channel between the two water bodies and just as the salinity in the lagoon began to fall to reach an average value of 44.5 psu (Azzati et al. 1987), demonstrates that the upper limit of tolerance of this bivalve is well above the previously described values (Schlieper et al. 1960, Butler et al. 1993). In the present work we verify that *P. nobilis* colonized the lagoon widely, withstanding a salinity range between 44 and 45 psu for several decades (1983-2014) before the eutrophication crisis occurred. Their preference for less saline areas within this range is likely to be due to the distance from colonization points rather than salinity in itself. The individuals in the Mar Menor population may grow to larger sizes than other populations because they are typically sheltered from detrimental hydrodynamics (García-March et al. 2020).

From the first moment of colonization until the beginning of the 2000s, the distribution of the individuals was marked by the distance to the entry points “Encañizadas” and Estacio channels (DENC and DEST), probably because larvae enter through these points from the population located in the Mediterranean side and whose largest population was along a coastal strip of approximately 500 m from the coast (DSH) and at 4 m depth (DEPTH).

The distribution of *P. nobilis* is usually patchy (de Gaulejac & Vicente 1990, Butler et al. 1993) and it is known that recruitment is influenced by environmental factors that synchronize spawning (Philippart et al. 2003; Cabanellas-Reboredo et al. 2009). Premise that is currently being analysed in the Mar Menor by these researchers. In the lagoon the specimen’s size inside a group seemed fairly homogeneous, which suggests that recruitment occurred in pulses, probably associated to key events of favourable environmental conditions in the lagoon. Rising seawater temperatures could affect recruitment (Philippart et al. 2003). Fluctuating temperatures, which are common in the lagoon, can be determinant in the reproductive processes of *P. nobilis*, and it is important to observe the relationship between environmental factors such as temperature and fan mussel spawning and recruitment periods (de Gaulejac 1993, Richardson et al. 1999).

During the first decades, after the entry of the first specimens into the lagoon, the connection channels with the Mediterranean (Estacio and Encañizadas) appeared to be an important entry pathway for larvae coming from the large population of fan mussel existing at that time in the Mediterranean *Posidonia oceanica* meadow in front of the lagoon. After the colonization resulting from larvae coming from the Mediterranean, during 2013-2014 the specimens reached reproductive maturity and began to colonize areas farther from the points of entry and the coastline (DSH) and the optimal depth of population distribution increased because they colonized deeper areas (DEPTH). Of interest is the fact that one of the main factors that explains the population in 2013-2014 is the presence of gravel. The high correlation between the distribution of fan mussel and the presence of high levels of gravel (GRAV) in the sediment can be explained by the fact that although it is common in sandy areas (Katsanevakis 2004), it needs to be attached by its numerous byssus filaments to large particles, seagrass rhizomes or solid substratum structures (Katsanevakis & Thessalou-Legaki 2009; Basso et al. 2015b). The Mar Menor is characterized by the presence in many areas of a high concentration of particles such as gravel of conchiferous origin and many of the specimens in the lagoon are attached to *Cymodocea nodosa* rhizomes but also *Bittium reticulatum* or others fragments of shells (García-March, 2003).

In the present work, a highly significant inverse relationship has been described between the potential distribution of the bivalve until 2016 and the distance to the higher eutrophic and hypoxic point in the lagoon (ALB: the mouth of the Rambla del Albujón). Although this variable has been removed from the models after detecting a high collinearity in the VIF analysis with the XCOOR variable and the texture variables (SAND; SILT). The mouth of the Rambla del Albujón (ALB) is a point where the oxygen reached an average value of 5 mg O_2L^{-1}, and minimum values of 0.47 mg O_2L^{-1} (Velasco et al. 2006). Even some studies have demonstrated that juveniles of *P. nobilis* are relatively resistant to moderate exposure to hypoxia (3.07 mg O_2L^{-1}) (Basso et al. 2015a).

However, all the processes of colonization and establishment of *P. nobilis* in the lagoon were interrupted by two simultaneous events in the Mar Menor area and the Mediterranean during 2016: i) the dramatic episode of eutrophication that caused a high mortality of specimens and; ii) the mass mortality event of *P. nobilis* in the south western Mediterranean Sea that caused the disappearance of the important population that for years had been an important source of larvae towards the lagoon.

A survey conducted between April and June 2017 confirmed a mortality of more than 90% of adult individuals in the lagoon. Two different situations can be observed. First, no living individuals were found below 2.5 m. in depth, even in areas where, until 2014, there had been a strong likelihood of individuals appearing (Fig. 9). This depth coincides with that described by Belando et al. (2017), which confirmed the total disappearance of benthic macrophytes below an average of 2.5 m depth in the whole lagoon, after the eutrophication episode of 2016 and its replacement by extremely slimy and anoxic sediment. It is highly probable that in these areas, the loss of light produced by the high concentration of phytoplankton in the water column was responsible for the death of the macrophytes, while the decomposition of the organic matter led to intense anoxia processes that caused the death of the macrofauna, including *P. nobilis*.

However, in the population located above 3 m depth, a survival rate of 10% was estimated (Fig. 9), which is higher than that described for populations affected by *Haplosporidium* in the Mediterranean (Vázquez-Luis et al. 2017). Although it is necessary to carry out many more tests on the survivors, during 2017 only one analysis of PCR was performed to a lagoon individual to and it was negative to the presence of *Haplosporidium pinnae* (Catanese et al. 2018). But no analysis of mycobacterium has been carried out in that period. No infective cells were detected in the digestive gland or hemocytosis that might suggest the existence of infection. However, an infection of the gills by ciliated protozoa (saprophytic opportunistic parasites) was observed, possibly as a consequence of the existing high organic load. To carry out tests to detect mycobacterium could help to resolve many doubts about mortality in the lagoon. Despite the significant decline in population due to the lagoon's environmental collapse in 2016, the stock of survivors is sufficient to begin a captive breeding program. The problem is that four years after the eutrophication crisis, the situation has not improved. Assuming that the environmental conditions of the lagoon will be restored, the preliminary data of this research could help make the Mar Menor an important reservoir of *P. nobilis* in the western Mediterranean. The lagoon is one of the few remaining sites where fan mussels still survive the disease, so maintaining good environmental conditions and healthy fan mussel populations should be a priority. The initiatives currently promoted by the responsible administrations, aimed at decreasing the levels of nutrients in the lagoon and consequently seeking an environmental recovery, have not currently been implemented. The survival of the species in the lagoon depends on its good environmental condition. Therefore, the application of the necessary measures for the recovery of the lagoon and the restoration of the fan mussel population is a priority and urgent. At the same time, it is important to continue with ex situ conservation actions and projects to achieve breeding in captivity.

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7. TABLES

Table 1. Main characteristics of the information sources used

Data-set	Campaign (year)	Method	Source	Number of presence points	Number of absence points	Total number of points
Historical	1983	Visual	Rodríguez Babio & Navarro Tarrega (1983)	1	0	1
	2003-2004	SCUBA diving; Snorkel transects; bathyscaphe	CARM (2003)	34	49	83
Integrated 2013-14	2013	SCUBA diving; Aquaplane; Bathyscaphe	Specific survey	31	29	60
	2014	SCUBA diving	Belando et al. (2014)	21	36	57
2016	2016	SCUBA diving	Specific survey	11	6	17

8. FIGURES

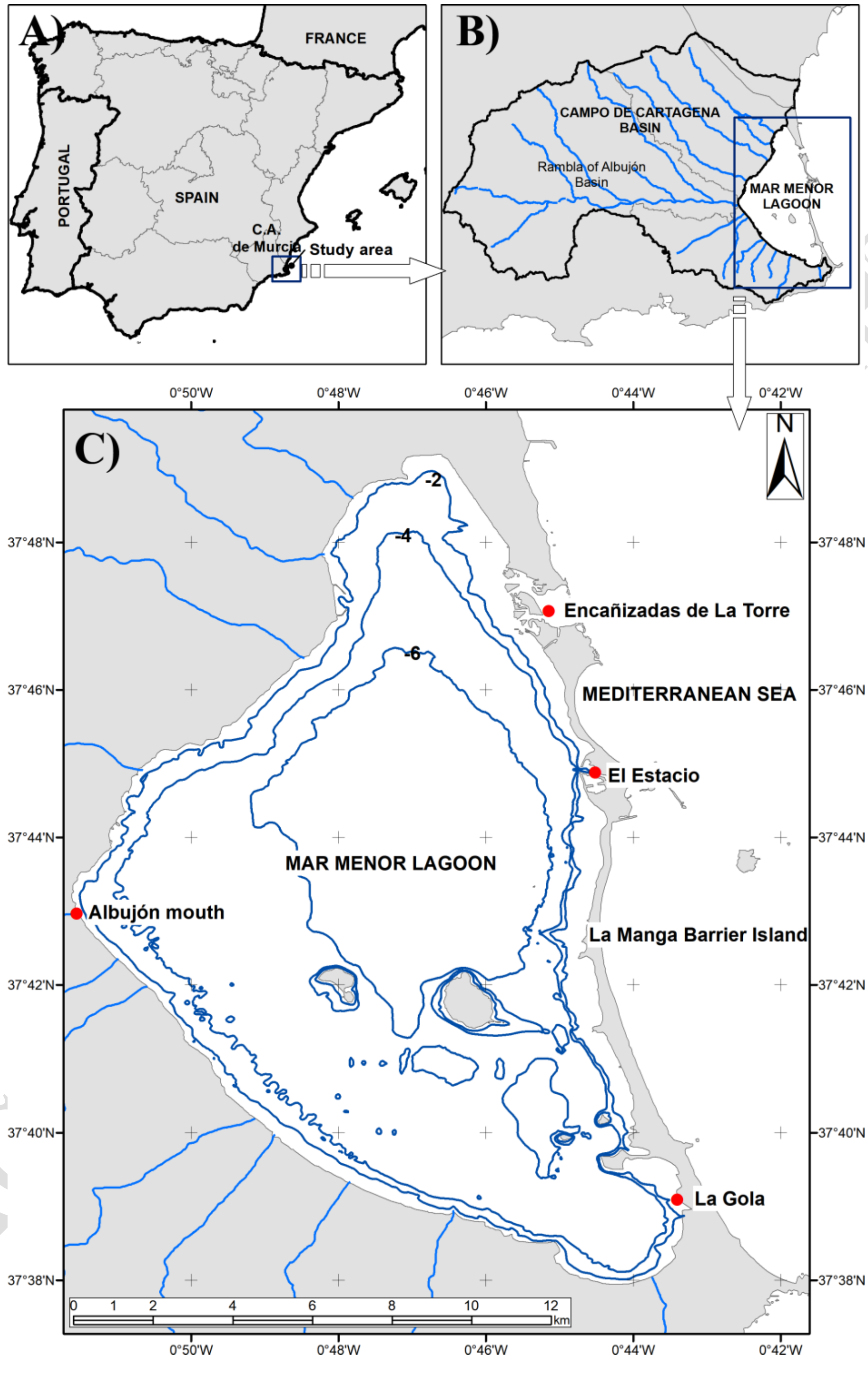

Fig. 1. Location of Mar Menor coastal lagoon.

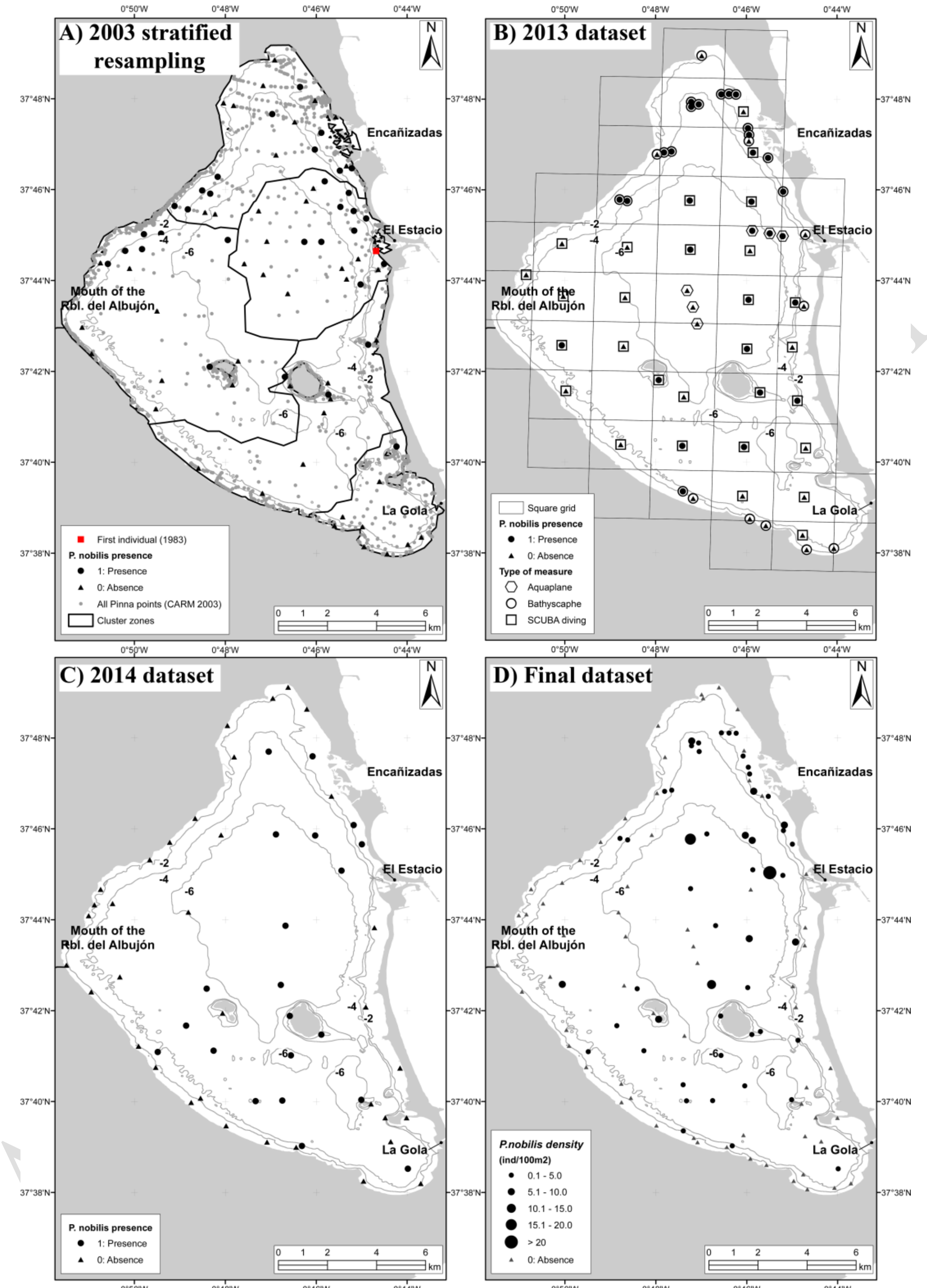

Fig. 2. Sampling survey. (A) 2003 data (Rodríguez Babio & Navarro Tarrega 1983, CARM 2003), including all points, the points obtained by resampling and the 5 cluster areas used to resample, obtained using the hierarchical cluster-based method; (B) 2013 data (specific campaign conducted in this research); (C) 2014 data (Belando et al. 2014); (D) Integrated 2013-14 data-set.

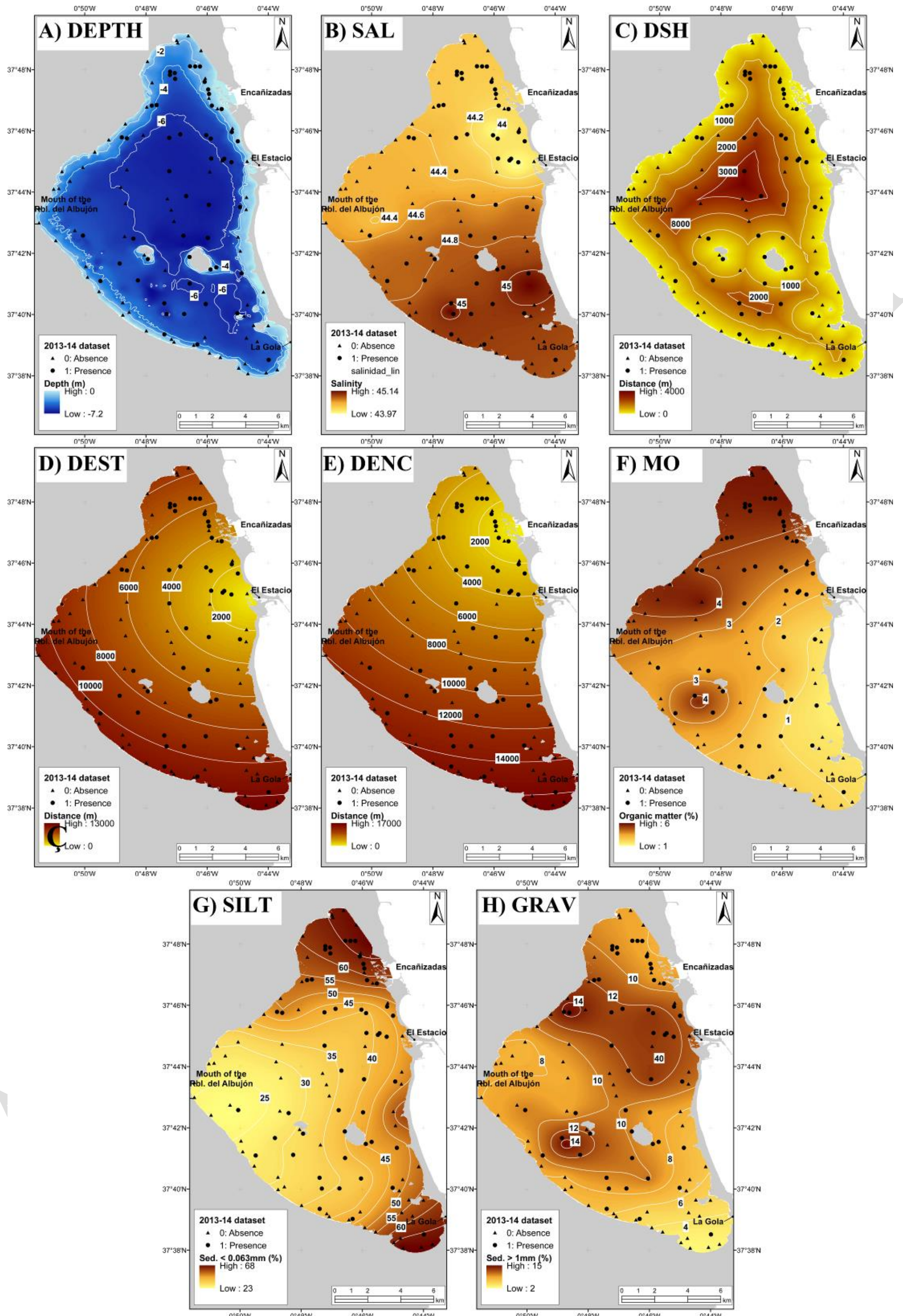

Fig. 3. Environmental variables used as predictors in the models. The nine variables selected after multicollinearity filtering are included. Acronyms in section 2.3 (Environmental data).

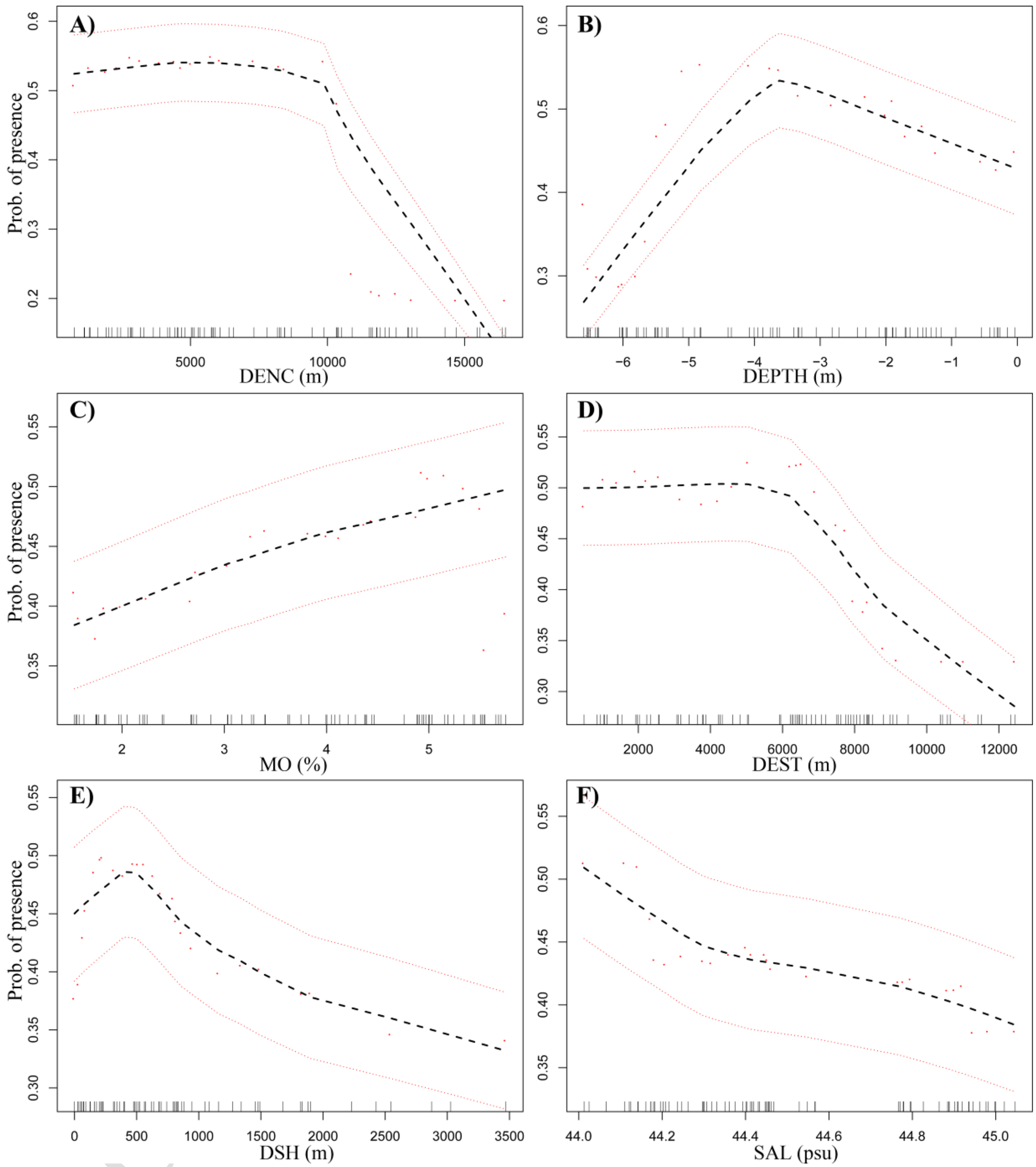

Fig. 4. Effects of predictors in the Random Forest presence-absence model with the variables selected by RFE. 2003-04. The variables are ordered from highest to lowest importance in the model. The rest of the variables are not considered relevant. Acronyms in section 2.3 (Environmental data).

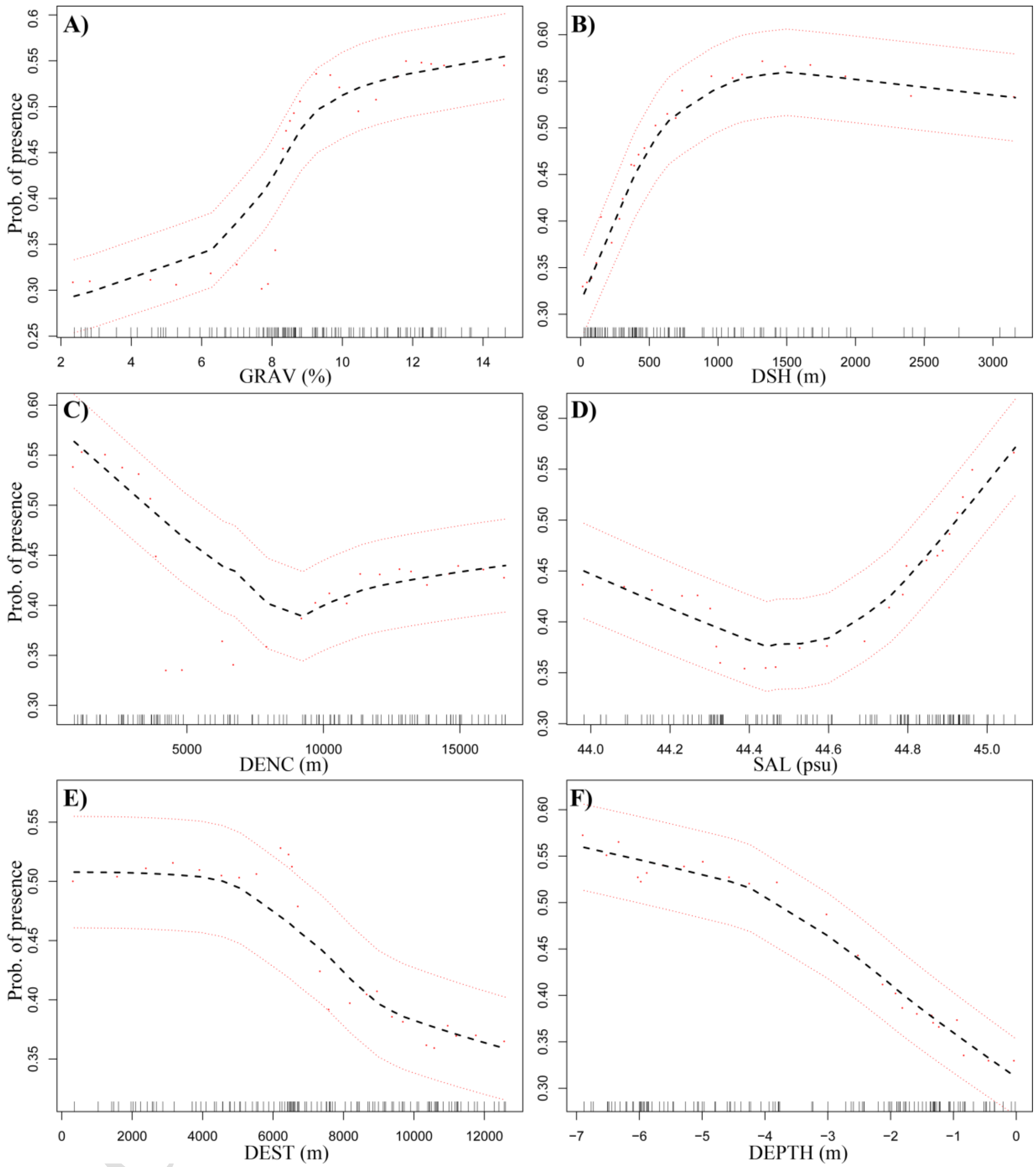

Fig. 5. Effects of predictors in the Random Forest presence-absence model with the variables selected by RFE. 2013-14. The variables are ordered from highest to lowest importance in the model. The rest of the variables are not considered relevant. Acronyms in section 2.3 (Environmental data).

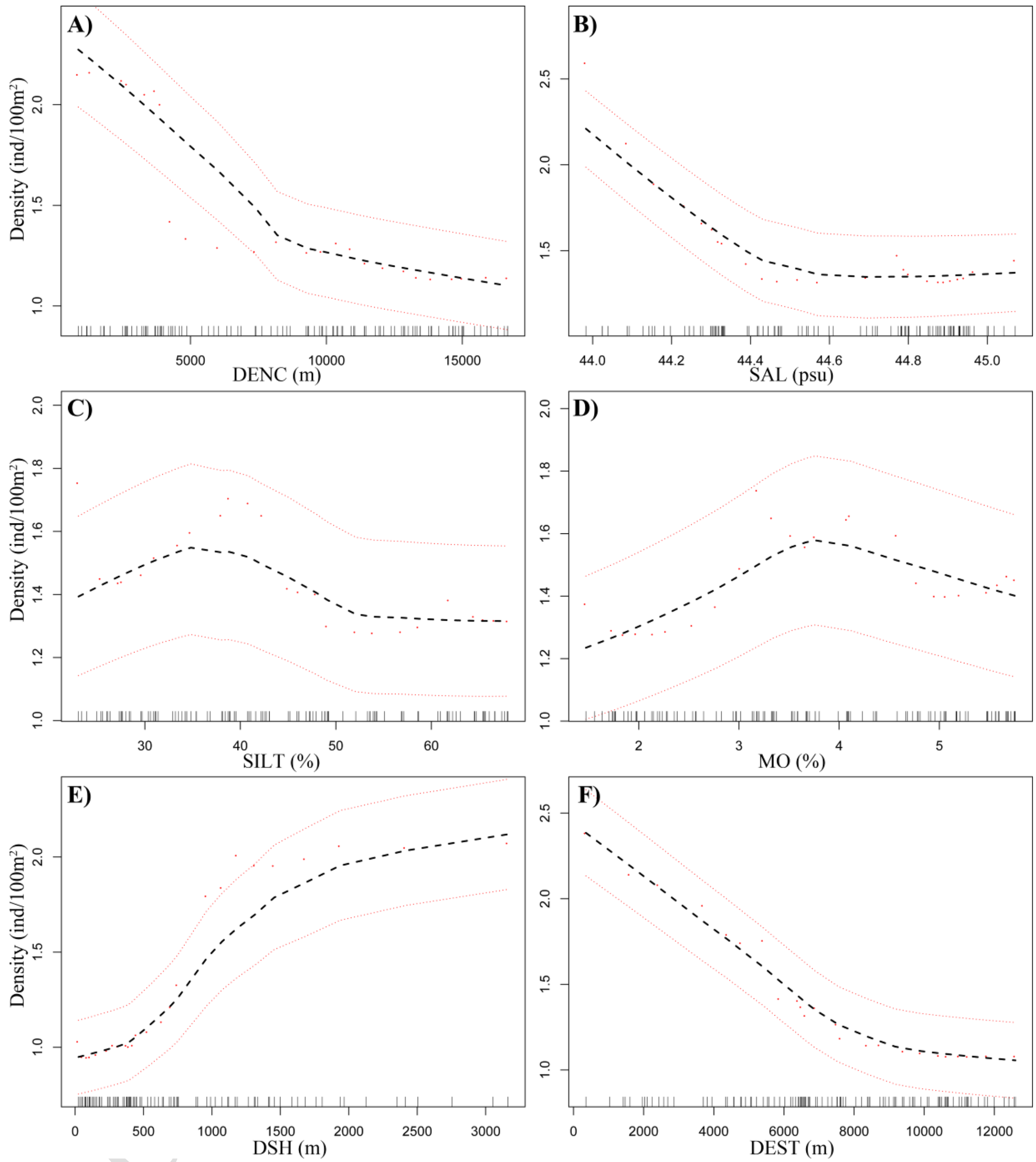

Fig. 6. Effects of predictors in the Random Forest population density model with the variables selected by RFE. 2014. The variables are ordered from highest to lowest importance in the model. The rest of the variables are not considered relevant. Acronyms in section 2.3 (Environmental data).

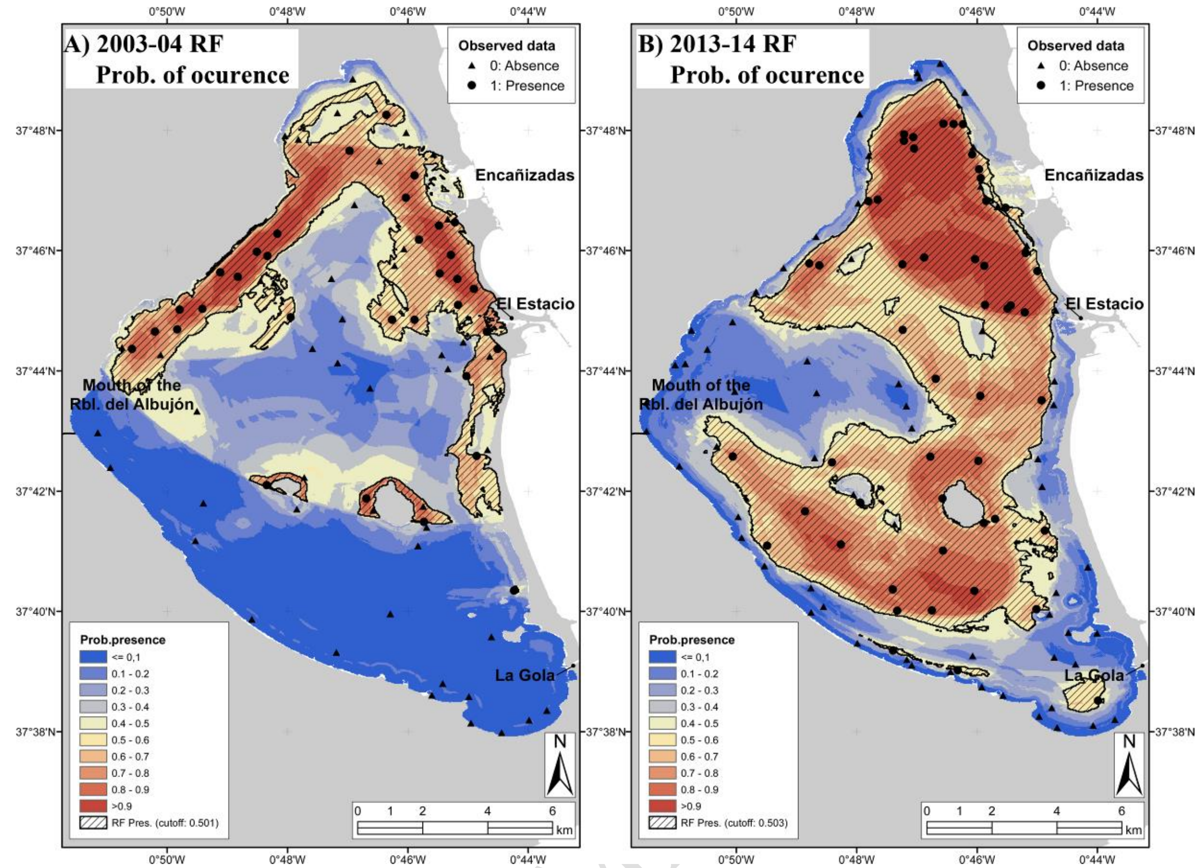

Fig. 7. Spatial prediction for *P. nobilis*. (A, B) potential distribution for *P. nobilis* in 2003-04 and 2013-14; presence-absence RF models with variable selection; line-shaded areas refer to potential areas (probability of occurrence above the cutoff point). (c) Spatial prediction of density for *P. nobilis*, 2013-14 data-set; RFRK model with variable selection using potential areas in 2013-14 as mask.

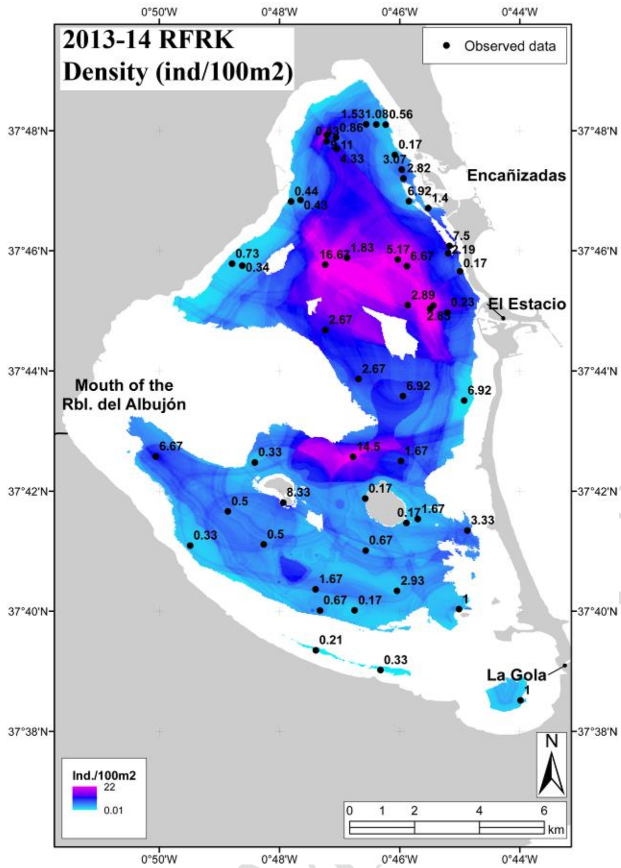

Fig. 8. Spatial prediction of density for *P. nobilis*, 2013-14 data-set; RFRK model with variable selection using potential areas in 2013-14 as mask.

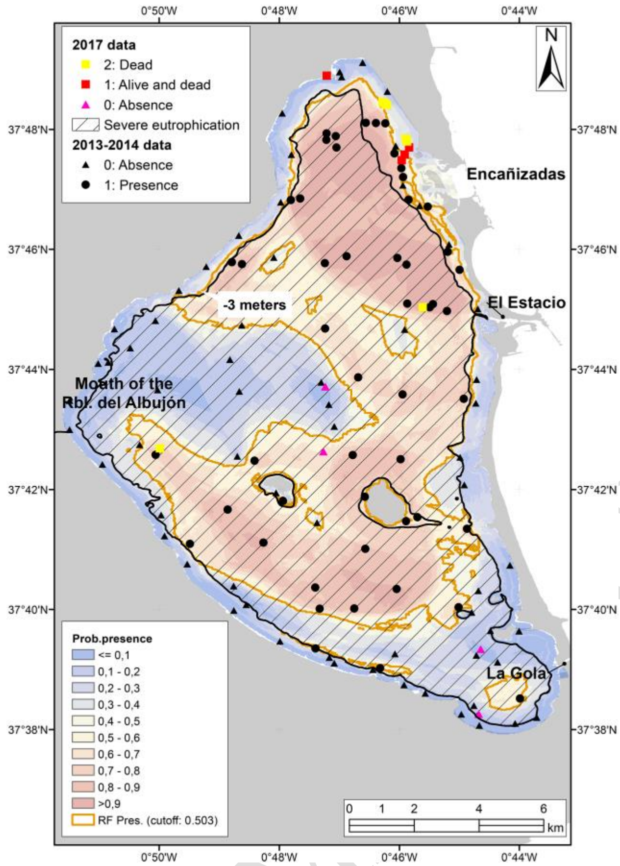

Fig. 9. Potential distribution in 2013-14 (presence-absence RF model and potential areas -probability of occurrence above the cutoff point-) versus 2017 data. Data sampled in 2017 (red and yellow squares, pink triangles) and data sampled in 2013-2014 (black dots and triangles) are included. The "severe eutrophication" area (line-shaded areas) refers to areas below -3 m in depth.