



The relict population of *Pinna nobilis* in the Mar Menor is facing an uncertain future

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ABSTRACT

Pinna nobilis is undergoing one of the most dramatic events suffered by an endangered species. An emerging disease has relegated its populations to coastal lagoons or estuaries with salinities beyond the 36.5–39.7 psu range. The Mar Menor is one of two such locations on the Spanish coastline. Poor environmental conditions and eutrophication and anoxia events, that became critical in 2016, 2019 and 2021, have reduced its population in >99 %. In this work, the spatial distribution of the species within the lagoon and the factors determining its survival along the successive crises of eutrophication are studied using a two-stage (presence/absence estimation and density modelling) Species Distribution Model. A potential area of 200.97 ha and an average density of 1.05 ind.100 m² is estimated for 2020. The viability of the Mar Menor population depends on management actions designed both for the species and to improve the lagoon environmental state.

1. Introduction

Pinna nobilis Linnaeus, 1758 is a Mediterranean endemic filter-feeding mollusc, one of the largest bivalves of the world, growing up to 120 cm (Katsanevakis, 2007; Zavodnik et al., 1991), and a good example of an ecosystem engineer species that filters large amounts of detritus, which gives it a high bioremediation power that contributes to water clarity (Rabaoui et al., 2015; Trigos et al., 2014).

It is settled in coastal areas mostly in soft sediments overgrown by meadows of seagrasses: *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera marina* or *Zostera noltii* (Zavodnik et al., 1991) or sandy bottoms, but also present in other habitats like maerl or rocky substrata (Kersting and García-March, 2017). Its salinity tolerance range was defined between 35 psu and 42 psu by Butler et al. (1993), but new data has increased it to 30–45 psu (Giménez-Casaldueiro et al., 2020; Prado et al., 2021).

In 2016, a massive mortality event was detected in most of the populations of the Spanish Mediterranean coast (Vázquez-Luis et al.,

2017). The parasite *Haplosporidium pinnae* was pointed out as the main responsible (Grau et al., 2022); however, other pathogens, such as *Mycobacterium* sp. (Carella et al., 2019; Šarić et al., 2020) and/or *Vibrio* sp. (Lattos et al., 2021; Prado et al., 2021; Rodríguez et al., 2017) could have acted synergistically. Such event eradicated almost all populations in the Spanish Mediterranean coast (Cabanelas-Reboredo et al., 2019; Catanese et al., 2018; García-March et al., 2020), changing its status at the Spanish national level, Orden TEC/1078/2018 (MITECO, 2018) from “Vulnerable” to “Critically endangered”. It has also been declared critically endangered on the International Union for Conservation of Nature Red List of Threatened Species (Kersting et al., 2019). In successive years, the pandemic spread to practically the entire Mediterranean; however, coastal lagoons seem to be places where this species resists infection (Čižmek et al., 2020; Katsanevakis, 2019; Katsanevakis et al., 2022; Kersting et al., 2019; Panarese et al., 2019; Ruitton and Lefévre, 2021). These sites are of particular importance, not only as reservoirs for the species, but also as potential sources of larvae to help recover other

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populations (Kersting et al., 2020).

In summer of 2016, a high mortality was observed in the *P. nobilis* population inside the lagoon; however, the cause did not seem to be the *Haplosporidium pinnae* epidemic (Giménez-Casaldueiro et al., 2020), but the devastating consequences of the environmental collapse occurring in the lagoon since the second half of 2015 (CACMM, 2017; Ruiz et al., 2020). A eutrophication crisis and a Harmful Algae Bloom (HAB) event took place after decades of nutrient and phytosanitary inputs from nearby agricultural activities (Aguilar-Escribano et al., 2016). In the spring of 2016, the high concentration of phytoplankton prevented the entry of light, which inhibited photosynthesis causing the death of the bottom vegetation below 3 m depth (Belando et al., 2017). The new environmental conditions, hypoxia even anoxia at some points, caused the massive mortality of many benthic organisms, among them a high percentage of *P. nobilis* individuals (Giménez-Casaldueiro et al., 2020).

In October 2019, after several months of massive phytoplankton

growth, an extreme rainfall event produced a massive freshwater discharge from the terrestrial basin leading to water stratification. This event generated an euxinic process (Ruiz et al., 2019) as the decomposition of organic matter by the proliferation of anaerobic bacteria in the deep water generated toxic compounds such as sulfides and methane, wiping out all existing life below 3 m depth (Giménez-Casaldueiro and Martínez-Fernández, 2020; Ruiz et al., 2019; Sandonnini et al., 2021b). This anoxic and toxic water mass emerged in the northern area of the lagoon on October 12, 2019, due to the effect of the easterly winds, causing the death of most of the macrofauna, including the pen shell colony geolocated in Villananitos beach (San Pedro del Pinatar). It has been estimated that an area >9000 ha has been devastated again in the lagoon bottom (Giménez-Casaldueiro and Martínez-Fernández, 2020; Ruiz et al., 2020; Nebot-Colomer et al., 2021).

The main objective of this study was to characterise the *P. nobilis* population in the Mar Menor lagoon after the environmental collapse of

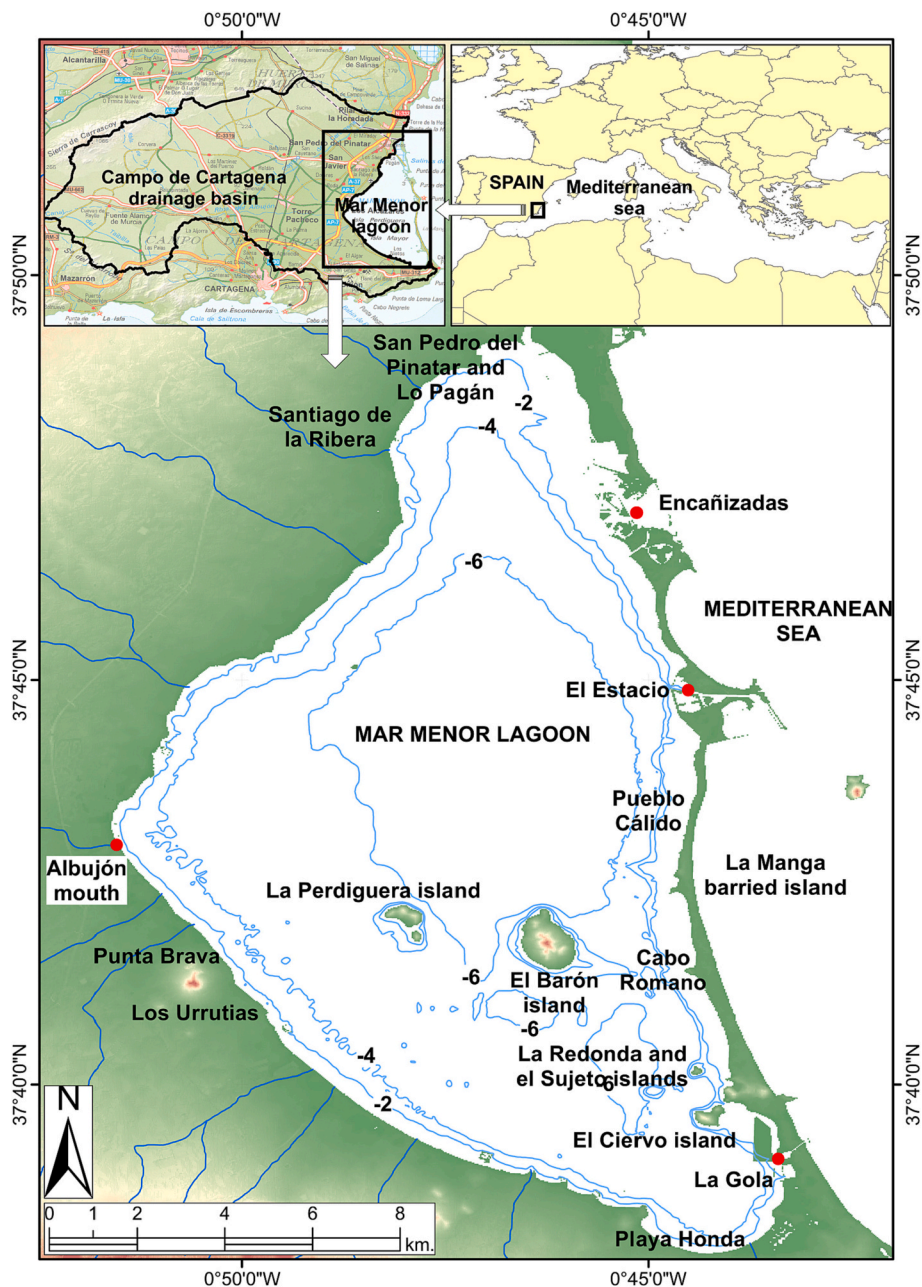


Fig. 1. Location of Mar Menor coastal lagoon. Source: BTN25 2006–2019 CC-BY 4.0 ign.es (administrative data); Derived data from MDT25 2015 CC-BY 4.0 ign.es; DGSCM (2009) (bathymetry).

2016 and identify the main factors that currently describe the distribution of the species using Random Forest Species Distribution Models (SDMs). This paper also continues the spatio-temporal study of the two periods prior to 2016 (2003–2004 and 2013–2014) carried out in Giménez-Casalduero et al. (2020).

2. Materials and methods

2.1. Study area

The Mar Menor is a 135 km² coastal lagoon in the South-western Mediterranean Sea (37°42'00"N 00°47'00"W), in a semi-arid region of Southeast Spain (Fig. 1). It is hypersaline (38–47 psu), warm and with extreme seasonal and daily temperature changes (8–32 °C). The mean depth is 3.5 m reaching >7 m. The lagoon is the main collector of the *Campo de Cartagena* drainage basin (Fig. 1) and is separated of the Mediterranean by a sandy bar, called *La Manga*, crossed by three shallow channels (López-Bermúdez et al., 1981). Its isolation and other geomorphological features cause particular environmental conditions such as temperature stress and high salinity, consequently it is dominated by euryhaline and eurythermal species (Mas et al., 2017). In the Mar Menor coastal lagoon, in 1976, new artificial channels between the lagoon and the Mediterranean led to hydrodynamic changes that altered the water renewal rate, decreasing the salinity values to 45 psu (Lloret et al., 2005) and allowing the settlement of the first specimens of the fan mussel (Murillo and Talavera, 1983; Rodríguez Babio and Navarro

Tárrega, 1983). In a few years, it became a key species within the Mar Menor, colonizing 65 % of the surface until 2014 (Giménez-Casalduero et al., 2020).

2.2. Species distribution models (SDMs)

We use the same approach used in Giménez-Casalduero et al. (2020) to analyse the consequences of the recent events on the *P. nobilis* population in the Mar Menor. The Species Distribution Models (SDMs) are statistical models of species–environment relationships based on location of individuals and environmental predictors that affect its distribution (Elith and Franklin, 2017) to explain observed patterns. SDMs are increasingly used in the marine environment; Guillaumot et al. (2021) and Robinson et al. (2017) reviewed and evaluated extensively various of these methods and their application to the marine environment. Martin et al. (2014) apply it for coralligenous and maërl habitats in Mediterranean basin; and Lauria et al. (2017) to investigate the distribution of two critically endangered octocorals in the central Mediterranean Sea. They propose a preferential habitat model (also known as delta model) based in a two-part Generalized Additive Model (GAM): an occurrence model using presence-absence data (family binomial), and a presence-only model using a Gaussian distribution; the final preferential habitat model is obtained multiplying the predictions from both models. More recently SDMs have been used to assess the impact of Climate Change on marine species (Allyn et al., 2020; Roberts et al., 2022).

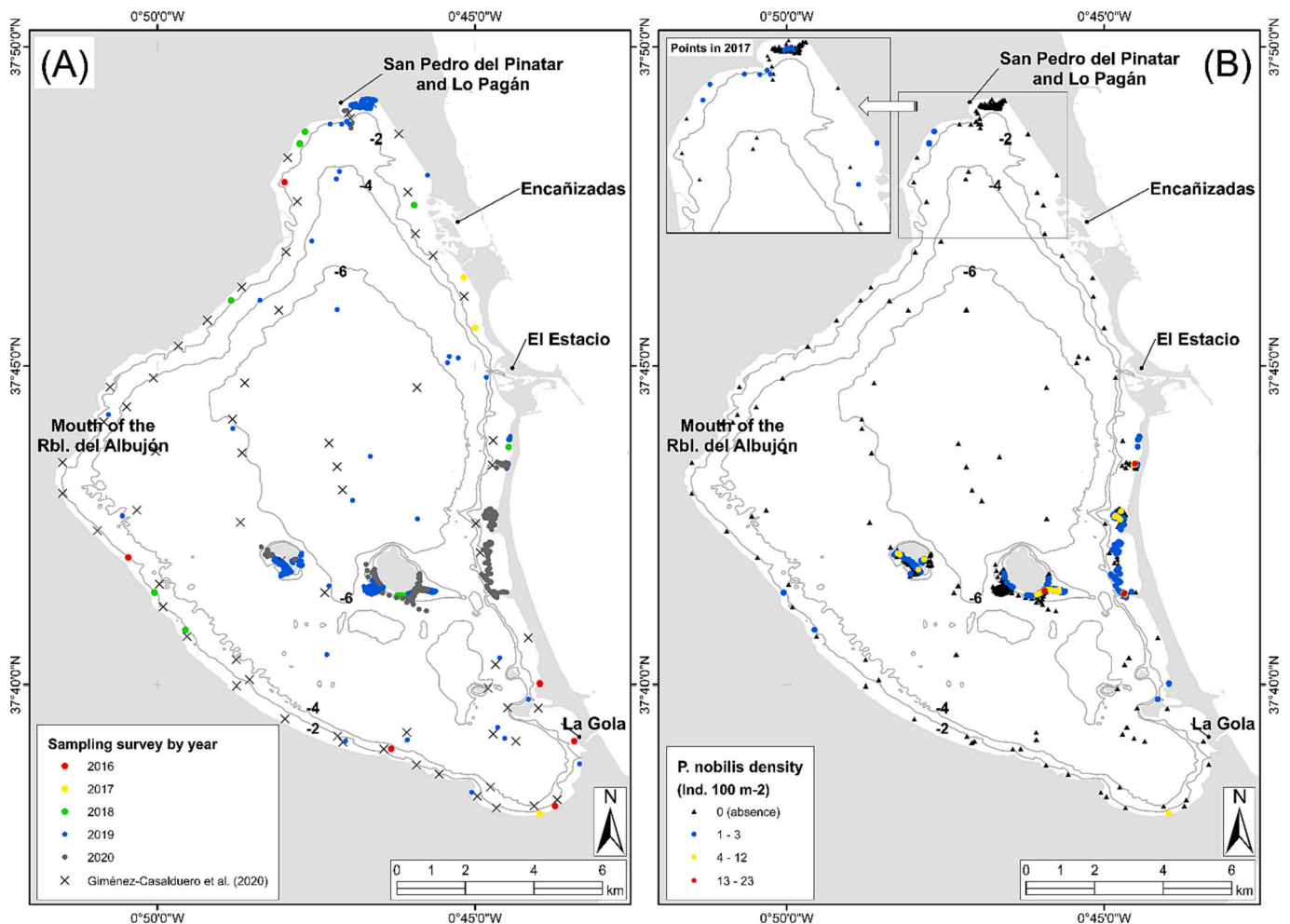


Fig. 2. (A) Sampling survey and (B) final dataset obtained as a result of its processing. The zoomed map includes the presence of *P. nobilis* in 2017 in Lo Pagán and Encañizadas that disappeared in 2020.

2.3. Data-sets and sampling methods

Data from 101 sampling days and 267 visual census between 2016 and 2020 (Fig. 2(A)) were collected using three approaches:

A) Eighty-two line transects (LT) were drawn in a range of 0–3 m depth (Katsanevakis, 2007), including the coastline of the main islands *El Baron*, *La Perdiguera* and *El Ciervo* (Fig. 1). All specimens located within 3 m from the line were counted and georeferenced. The lines were recorded by a Global positioning System (GPS GARMIN eTrex 20× and Montana 680 t); B) A modified linear transect (MLT) methodology (Siletic and Peharda, 2003) was used in order to survey the *P. nobilis* population density and size; it consisted of a 50 m length and 3 m wide transect (total area per transect of 150 m²). A total of 121 MLT in two complementary activities were used at different depths: i) Complementary campaigns were conducted between 0 and 3 m within a citizen science activity to complete the information obtained with the LT samplings. Four surveys were carried out in three locations (*San Pedro del Pinatar*, *Santiago de La Ribera*, *Cabo Romano*) with a total of 43 visual census and the specimens found were measured (García-March and Ferrer, 1995). Before the sampling activity, a methodological training talk was given to participants by the specialized staff. ii) An intensive MLT sampling (IS) was carried out in two localities (*El Baron* and *La Perdiguera* islands) to identify new specimens and to analyse the *P. nobilis* population changes in depth and size. Two SCUBA divers conducted a total of 78 linear and underwater transects perpendicular to the shore, counting live and dead specimens within each quadrat with direct visual observations between 2.5 and 6 m deep. Density and size were counted for both live and dead specimens. For each individual the maximum length shell was recorded (García-March and Ferrer, 1995); C) To complete the spatial information of *P. nobilis* absences, 64 points sampled in Giménez-Casalduero et al. (2020) were added after checking that *P. nobilis* was still absent.

Once the locations were obtained using the previous methods, the processed data were added to regular cells of 10 × 10 m, obtaining as a result counts of *P. nobilis* every 100 m². The output is a number of presence records per unit area as a measure of abundance rather than probability (Renner et al., 2015). This final dataset has been used to calibrate and validate the SDMs (Fig. 2(B)), computing a total of 845 points, 389 with presence of *P. nobilis* and 456 of absence, and a maximum value of abundance of 23 ind. per 100 m⁻² on *El Baron* Island. However, to study the evolution from 2017 to 2020, the presence points in *Lo Pagán* (9 points) and *Las Encañizadas* (2 points) (zoom in the Fig. 2 (B)), whose individuals had disappeared in 2020, have been included.

The antero-posterior length outside the substratum (*Hs*) was measured. Total shell height (*Ht*) was estimated from the maximum width of the valves (*lc*) using the following equation (García-March and Ferrer, 1995):

$$Ht = 1.79 \cdot lc + 0.5 \pm 0.2$$

An additional count of the dead specimens, including the shell size was made.

The frequency distribution of density for size class and depth was studied using their skewness and kurtosis. Differences in the size distribution and density among dead and alive individuals were tested with a *t*-test (significance level = 0.05), based on a contingency table of the size classes and the habitat types (Zar, 2009). Summary and statistical analyses were performed using PAST software (Hammer et al., 2001).

2.4. Modelling framework

The modelling framework consists of a two-stage SDM in which presence/absence is first estimated with a binomial classification model in which the probability threshold to split presence and absence is optimised according to the Youden index method. Density is then modelled (regression model) within the presence areas identified by the previous model. This type of framework has been used with good results

by authors such as Lauria et al. (2017) or Allyn et al. (2020), who highlight two advantages: a) the two-stage approach accommodates situations where the number of absence observations exceeds those expected from traditional “count” distributions, obtaining better fit and b) it does not require a priori assumptions and can reproduce non-linear relations. The methodology described was implemented with R software (R Core Team, 2022). This approach allows one to obtain both probability of occurrence and density at the same time, and also the effects of the predictors.

2.4.1. Environmental data and variable selection

Eleven spatially distributed environmental variables were initially considered (Figs. S1 and S2 of the supplementary material).

Six time-static auxiliary variables are included (Figs. S1 and S2): i) DSH: Distance to the shore (m); ii) DEPTH: Depth (m), obtained by spatial interpolation (Ordinary Kriging) from bathymetric curves and depth points available from the eco-cartographic studies (DGSCM, 2009); iii) DEST: distance to *Estacio* channel (m); iv) DENC: Distance to *Las Encañizadas* channel (m); v) DMAR: Distance to *Marchamalo* channel (m); vi) DALB: Distance to the *Rambla del Albuñón* mouth (m).

Five dynamic variables were averaged for 2017 and 2020: vii) CHL: chlorophyll concentration (mg/m³); viii) TURB: turbidity (nephelometric Turbidity - NTU); ix) O: dissolved oxygen (ml/l); x) SAL: salinity (PSU) and xi) TEMP: sea temperature (°C). These surface variables were obtained from the UPCT (2022) at the L2 level.

The number of predictors in the SDMs was reduced using two complementary approaches: A general evaluation of the multicollinearity between predictors using the variance inflation factor (VIF) was carried out. The VIF of each of the 11 variables was first estimated against the rest and the variable with the highest value was eliminated; this process was repeated iteratively until the VIF of all the remaining variables were lower than 5 (Kutner et al., 2005). In addition, in the estimation process of each SDM, predictors were selected using a forward feature selection (ffs) (Meyer et al., 2019) based on Leave-Location-Out cross-validation (LLO-CV). The process begins by estimating all possible two-predictor models to select the most accurate according to a K = 10 folds LLO-CV (using AUC for the presence-absence models and RMSE for the regression models). Then all three-predictor models that include the previously selected two are tested, keeping the most accurate. The process continues iteratively until it stops when none of the remaining variables significantly increases the accuracy with respect to the current model.

2.4.2. Classification and regression models

Both presence/absence and density SDMs were obtained using Random Forest Regression-Kriging (RFRK), in a similar way to Hengl et al. (2007) Regression Kriging, but using Random Forest (RF) as global classification or regression method. This method is a framework based on two steps: It first, uses a regression or classification method globally to predict the spatial distribution; secondly, the residuals of such models are interpolated using ordinary kriging. This way, it is possible to integrate the global effects of the predictors with the spatial distribution of the variable with local autocorrelated effects. RF (Breiman, 2001) is a non-parametric machine learning method for classification or regression that builds an ensemble of trees. It uses two parameters: number of trees (n_{tree}), and the number of features randomly sampled in each split (m_{try}), although the algorithm is not very sensitive to their values (Liw and Wiener, 2002). We used $n_{tree} = 1000$, doubling the default value, and m_{try} was calibrated to maximise AUC in classification and to minimise RMSE in regression, following the cross-validation scheme (LLO-CV) set out in subsection 2.4.3. RF has several advantages (Gomariz-Castillo et al., 2017): it is a non-parametric and non-linear method that produces more accurate results than other classification-regression methods, even when most of the features are noisy, it is less prone than others to overfitting, giving more generalization capacity. In addition, it provides a metric of the predictors importance based on the accuracy differences when a predictor is or is not included in the model.

2.4.3. Validation of SDMs models

Standard out-of-sample cross-validation can be over-optimistic if data are not spatially independent, potentially leading models to overfit local patterns in the data and to a misinterpretation and overoptimistic view of the resulting models (Meyer et al., 2018; Roberts et al., 2017). Block resampling techniques can be used to solve this problem. This work has used a spatial block cross-validation buffering (LOO-BUF-CV) strategy implemented in Valavi et al. (2019), used in the marine environment by Smith et al. (2021) among others. This approach is similar to leave-one-out cross-validation (LOO-CV), where the number of folds equals the number of instances in the data set, using in each iteration a single point to validate the model and all other instances to calibrate it; it results in a reliable and unbiased estimation of model accuracy (Hastie et al., 2009). The difference with respect to LOO-CV is that LOO-BUF-CV generates spatially separated training and testing data by considering circular buffers of specified radius around each observation point, not using the points closest to the validation point as training set. This way the independence in cross-validation increases. In this studio, a radius of 500 m was used, both to validate the models and to select the covariates. To validate the presence/absence models, we used AUC function, overall accuracy (Acc) and kappa index. The threshold probability value to decide if the species is or is not present was calculated using Youden method that tries to maximise both specificity and sensitivity. To validate the abundance models, we used RMSE, coefficient of determination (R^2) and Kling–Gupta efficiency (KGE) (Gupta et al., 2009). The latter is perhaps the least known in this type of studies; it is interpreted very similarly to R^2 (ranging from $-\infty$ to 1) and summarizes correlation, bias and variability in a single index, solving some deficiencies of R^2 (Ruiz-Álvarez et al., 2021).

3. Results

During the monitoring campaigns of 2018 and 2019, specimens were found uprooted and knocked down during the setting and removal of fishing nets and even directly extracted. In one of the locations where this situation was observed, *La Perdiguera* island, the colony disappeared completely, possibly due to illegal fishing nets.

3.1. Population density structure

The average length of the 82 snorkelling line transects (LT) was 2314 ± 268 m with a track time of 93 ± 9 min. These transects, and the 121 modified linear transect (MLT), recorded a total of 686 fan mussel live individuals, 86 % of them were georeferenced.

During the intensive sampling (IS), 89 live and 69 dead specimens were found. Live individuals were located between 0.4 and 2.9 m depth, and 85 % of them were located between 1.1 and 2 m. The depth range observed for dead individuals was between 0.4 and 5.8 m, 71 % of them at depths larger than 4 m. In the depth range where live specimens were encountered (0–3 m), only 17.5 % of the specimens were dead, while below 3 m depth it was 100 % (Fig. 3).

Live specimen distribution by depth was slightly platykurtic with a kurtosis value next to 3 (g_2 : 2.8) and moderately negative skewness (g_1 : -0.69), but dead specimens (D) distribution by depth was extremely platykurtic (g_2 : -0.67) with a very negative skewed distribution (g_1 : -1.04 ; < -1).

Statistical analysis showed that the average depth of live specimens (1.65 ± 0.04 m) was significantly shallower than the average depth of dead specimens (4.48 ± 0.21 m) (t -test, $p < 0.001$). Dead specimens are generally found at greater depths, and there is no living specimen below the threshold 3 m (Fig. 4).

Size-class distribution of live and dead specimens of *P. nobilis* was analysed and significant differences (t -test, $p < 0.001$) were observed (Fig. 5). Dead specimens were bigger, with maximum size of 84.63 cm and average of 41.32 ± 1.66 cm, whereas 58.48 cm and 33.57 ± 0.82 cm were measured for the live ones. Both live and dead specimens

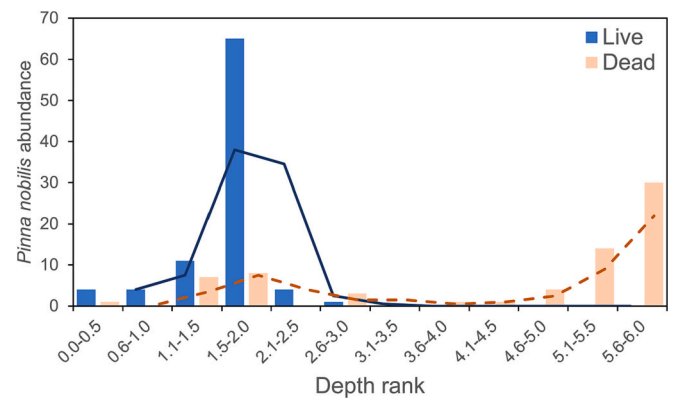


Fig. 3. Abundance distribution of live and dead *P. nobilis* individuals in different depth ranges. Lines coincide with the trend line from the moving average.

(Fig. 6) exhibited a moderately right-skewed body size–frequency distribution ($Lg1$: 0.61; $Dg1$: 0.88), and both were platykurtic ($Lg2$: 0.93; $Dg2$: 1.58).

3.2. Global evaluation of the models

Table S1 of the supplementary material summarizes the LOO-BUF-CV goodness-of-fit statistics obtained for the presence/absence and density models. To benchmark the results of RF and RFRK against other widely used methods, the validation results of a Generalized Linear Modes (GLM) and a Generalized Additive Model (GAM) are included, using logistic regression for presence-absence and Poisson regression for density. In GAM models, a cubic spline is used as smoothing function (Hastie and Tibshirani, 1990).

RFRK has the best fit both in presence-absence (AUC: 0.786, CI: 0.030) and density (KGE: 0.772, CI: 0.055), results very similar to those obtained using RF; meaning that most of the variability is addressed by RF, including spatial autocorrelation. Regarding the rest of the goodness-of-fit statistics, RFRK and RF stand out, with very high R^2 values in the density models and RMSE significantly lower than GLM and GAM. It is also noteworthy the very low R^2 values of GLM and GAM for the validation set, and negative KGE values in the case of the GLM-based models; such values indicate that these models are not adequate to predict the *P. nobilis* spatial pattern in the lagoon. Accuracy in the presence-absence models is not very high, but it may be underestimated considering that the validation system eliminates the points closest to each evaluated point, and that the current spatial pattern of *P. nobilis* in the lagoon is very concentrated on a few spots. In addition, both RF and RFRK in the final model (calibration stage) manage to classify well 100 % of the points in the final model, obtaining a KGE very close to 1.

3.3. Variable selection and effects of predictors

Seven variables were included in the models after VIF analysis: DEST, DEPTH, DENC, CHL, TURB, DSH and O. Fig. S1 of the supplementary material include Pearson correlation matrix of analysed variables in 2020; Figs. S2 and S4 show their spatial distributions.

Fig. 7 shows the variable importance in the RF models, the forward feature selection (ffs) analysis identified four variables as final variables to be included in the presence-absence and abundance models: DEST, DEPTH, DENC and CHL in the presence-absence models (Fig. 7(A)) and DEST, DEPTH, O and CHL in the density model (Fig. 7(B)). The most relevant variables to explain the distribution pattern were DEST and DEPTH. DENC is included in third place just in the presence-absence model, whereas O is the third variable in the density model. Lastly, CHL is the fourth variable in both models.

Figs. 8 and 9 include partial dependence plots that explore the effects

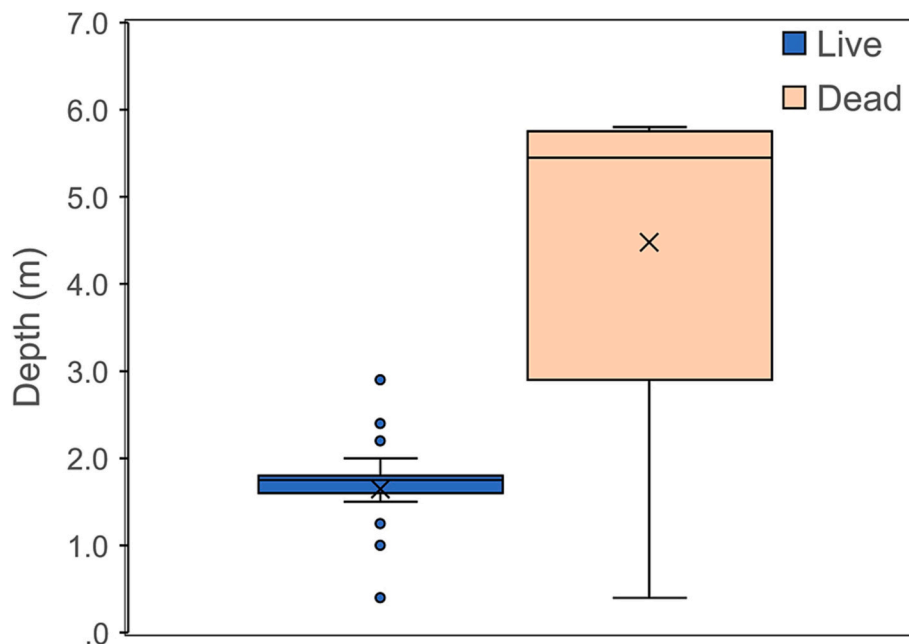


Fig. 4. Boxplot of depth of *P. nobilis* individuals live ($n = 89$) or dead ($n = 69$). The blade within the box marks the mean.

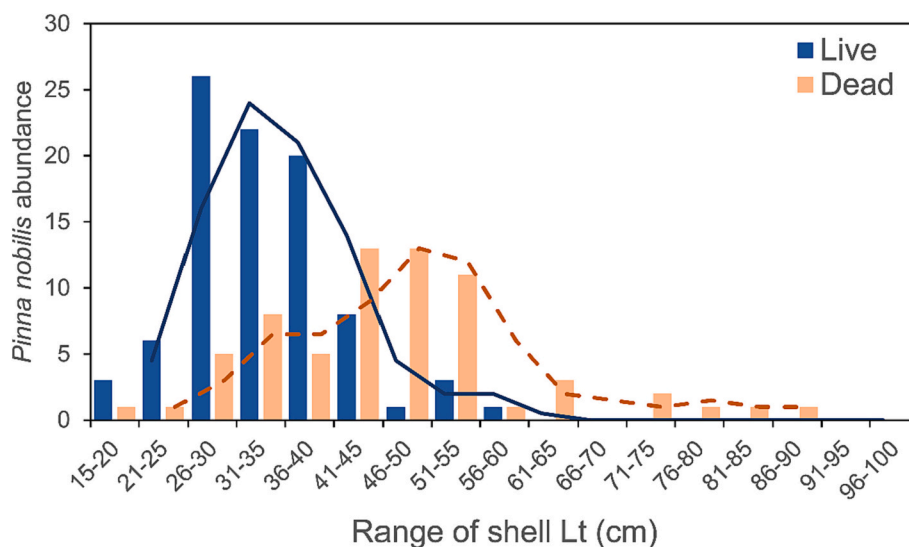


Fig. 5. Size class distribution of live and dead *P. nobilis* individuals in different depth ranges. The dashed lines coincide with the trend line from the moving average.

of the covariates on the presence probability (in the presence-absence model) and response (in the regression model). Fig. 8(A) shows how the maximum presence probability occurs at some distance from the *El Estacio* channel (DEST), coinciding with the populations of *La Manga* close to this communication channel, and reaches the maximum presence probability at about 7000 m distance, decreasing drastically from this threshold. Something similar can be seen in Fig. 9(A); interestingly, the density increases in the range 6000–7000 m, this distance coincides with the populations of maximum density in *El Barón* island. In the case of DENC in presence-absence (Fig. 8(C)) the pattern describes a linear increase in probability as we move away from *Las Encañizadas* until a maximum around 10,000 m, a distance that coincides with the populations of *La Manga*. This pattern is different in 2017, as in that year there was still an important population in the vicinity of *Lo Pagán* and *Las Encañizadas* and in the northern part of the lagoon (zoom in Fig. 2 (B)). Both population centres disappeared in 2018 and 2019 respectively.

The effect of DEPTH in presence-absence and density models is similar (Figs. 8(B) and 9(B)). The highest concentrations appear in the range -1.1 to -2.5 m, decreasing towards the coastline and more drastically as depth increases until reaching a clear limit at 3 m, as observed in Giménez-Casalduero et al. (2020) or Ruiz et al. (2020). Regarding physico-chemical variables, the presence of fan mussels drops for chlorophyll values higher than 3 ml/m^3 (Figs. 8(D) and 9(D)), but the maximum density is observed at levels close to this concentration, which could be due to the filtering capacity of the species (Davenport et al., 2011). Regarding Oxygen concentration (Fig. 9(C)), the values in depth of anoxic and/or hypoxic situations are not reflected because the variable used contemplates mean values for the studied period, not the specific episodes in which this situation occurred.

3.4. Spatial prediction of SDMs models

Fig. 10 shows the prediction in terms of probability of presence for

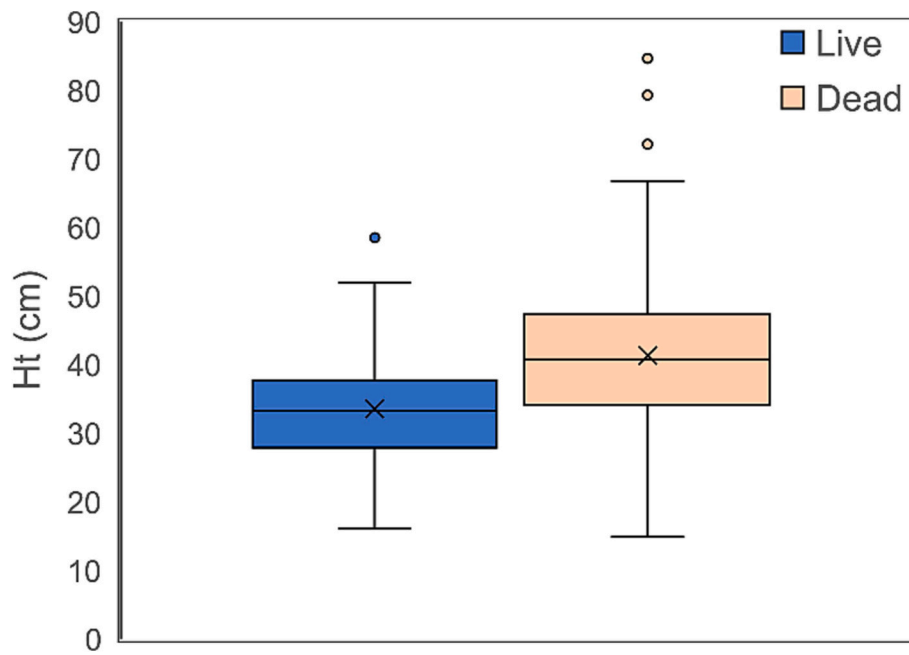


Fig. 6. Boxplot of Total shell height (*Ht*) of *Pinna nobilis* individuals live ($n = 88$) or dead ($n = 66$). The blade within the box marks the mean.

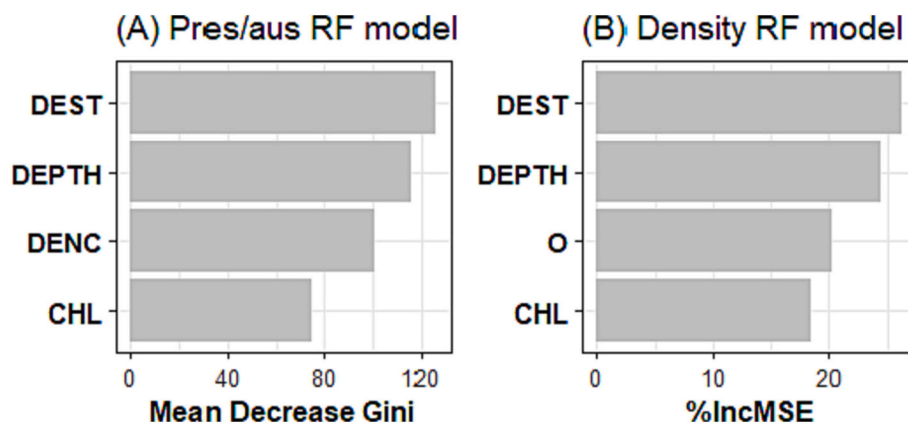


Fig. 7. Variable importance for (A) presence-absence and (B) density RF models. The values are de-escalated using the ratio mean/standard error.

(A) 2017 and (B) 2020 and the areas of potential presence (areas whose probability of presence is above the optimised cut-off point: 0.49). The spatial pattern is very similar in both cases, except for the drastic reduction between 2017 and 2020 in the north of the lagoon, in the area of *Las Encañizadas* (Fig. 8(C)), and *San Pedro del Pinatar* and *Lo Pagán* (zoom in Fig. 2(B)).

As a result, the area of potential occurrence in 2017 was 323.34 ha (2.40 % of the of the Mar Menor area), while in 2020 it is estimated about 200.97 ha (1.50 %). The area in the north of *El Barón* island, although not inventoried, is included in both dates as an area of potential occurrence of *P. nobilis* based on its predicted probability of presence.

In 2020 the potential areas, coinciding with the sampled points where *P. nobilis* is present, are identified in a strip of low depth near *La Manga*, from *El Estacio* to *El Ciervo* island (Figs. 8 and 9). The model also includes a certain increase in probability of presence in *La Gola*, although without reaching 0.495, almost connecting this area with the potential area identified in the south of the lagoon, in *Playa Honda*. Also noteworthy is the high probability of presence around *El Barón* and *La Perdiguera* islands, also in the range of depths identified in Figs. 10 and 11. Finally, two potential areas are identified, where a few individuals

were sampled, to the southwest of the lagoon, in *Punta Brava* and *Los Urrutias*, and in the area of *La Ribera* to the northeast of the lagoon, being the only individuals still alive from the group sampled in 2017 in the area of *San Pedro del Pinatar* and *Lo Pagán* (zoom in Fig. 2(B)).

Fig. 11 shows the density model prediction. The estimated average density in the presence area is 1.055 ± 0.006 ind. per 100 m^{-2} (mean \pm SE) with values varying between 0.01 and 23 ind. per 100 m^{-2} .

4. Discussion

The specimens of *P. nobilis* that colonized Mar Menor in the 1980s came from a large, nearby population in the Mediterranean (Giménez-Casaldueiro et al., 2020), a more adequate and stable environmental conditions in terms of salinity and temperature. They entered in a lagoon with more extreme seasonal temperature changes ($8 \text{ }^{\circ}\text{C}$ to $30 \text{ }^{\circ}\text{C}$ in shallower waters) and abrupt changes in surface salinity due to the input of fresh water during the rainy season and Mediterranean water during storms. This may explain why the first colonization area of *P. nobilis* was the more stable deepest zone, these specimens grew larger because they had been in the lagoon for much longer and had suffered less anthropic pressures. After years of adaptation, *P. nobilis* managed to colonise the

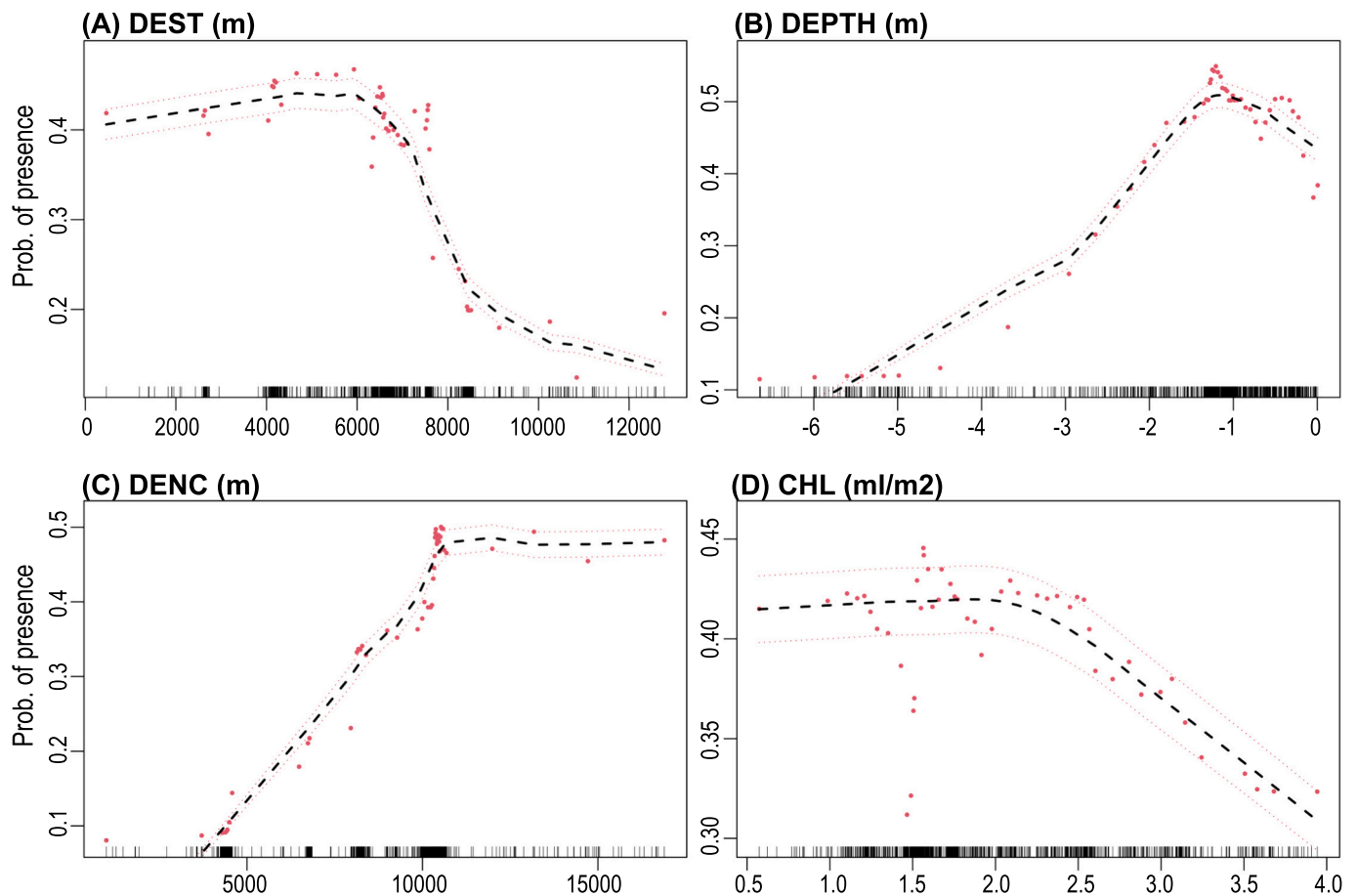


Fig. 8. (A–D) Effects of predictors in the RF presence–absence model with the variables selected by ffs. The variables are ordered from highest to lowest importance in the model (Fig. 7(A)). The black dashed line represents the estimated effect, the dotted red lines the 95 % confidence intervals, and the red dots indicate partial values for observed values. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

rest of the lagoon, even the shallowest areas (Giménez-Casalduero et al., 2020).

Our results show a patchy distribution, high density areas surrounded by areas without specimens; this behaviour has been described by different authors as a peculiarity of this species (Butler et al., 1993; García-March and Ferrer, 1995; Prado et al., 2022). The size of individuals within a group seem to be quite homogeneous, suggesting that recruitment in the lagoon occurred in pulses, probably associated with key events of favourable environmental conditions that synchronise spawning, like an increased seawater temperature (Philippart et al., 2003; Cabanellas-Reboredo et al., 2009; Kersting and García-March, 2017). Episodes of synchronised reproductive pulses have been observed in the lagoon associated with small changes in water temperature (Kersting and García-March, 2017; Gimenez-Casalduero, F. and Cortés-Melendreras, E., pers. Obs.). Since 2017, recruitment of juveniles has been observed inside the lagoon, some of which remain viable over time.

A depth-related size segregation in the Mediterranean, with smaller individuals and the absence of larger ones in shallower waters, has been reported (Barberá et al., 1996; Moreteau and Vicente, 1982). The specimen distribution in the Mar Menor, before the 2016 eutrophication crisis, corresponded to this pattern (Giménez-Casalduero et al., 2020). The dead specimens distribution, with a higher density and larger individuals in the deepest areas (between 5 and 6 m depth), is also in line with that pattern. This contrasts with the situation after 2016, when the average size of the surviving specimens was significantly smaller than those existing prior to 2015.

The fact that the specimens in the deeper areas of the lagoon are

larger and older may reflect the adaptation of the species to the optimal conditions in the lagoon before 2016, where the deep zones suffered less anthropic pressure (an 80 cm specimen that could be 30 years old was found in 2012). The absence of large specimens in the depth range of 0–3 m, may also be related to extractions by fishermen or tourists (Cortés-Melendreras et al., 2021). During the last decades, a dense population of *P. nobilis* has difficult to set and haul fishing nets in some areas. Likewise, the high density of specimens has been an attraction for tourists who collected them as curiosities, although this behaviour is not common (Richardson et al., 1999). The disappearance of the larger specimens, located in the deep areas of the lagoon, may have influenced the decrease in the reproduction rate of the Mar Menor population, although this fact seems to be more related to the drastic decrease in the number of breeding specimens than to their size, since in shallow areas there were also breeders in stages prior to the problems of eutrophication of the lagoon. Comparing the surface area of both potential areas with those estimated in Giménez-Casalduero et al. (2020) for 2003–2004 (2985 ha) and 2013–2014 (7385 ha), the recession of *P. nobilis* in the lagoon is clear.

The distribution pattern in 2021, compared to that of 2014, is limited to relict areas in a straitened depth range between 0.5 and 2.8 m, 90 % of the surviving specimens are concentrated in a narrow strip between 1 and 2 m at an average distance of 450 m to the coast. Specimens at distances larger than 1000 m from the coast are located on the islands (*El Baron* and *La Perdiguera*). The pattern observed in 2014 was the opposite, with a lower presence probability at the coastline that increased with the distance to the coast and stabilised at 1000 m in a 50 % (Giménez-Casalduero et al., 2020).

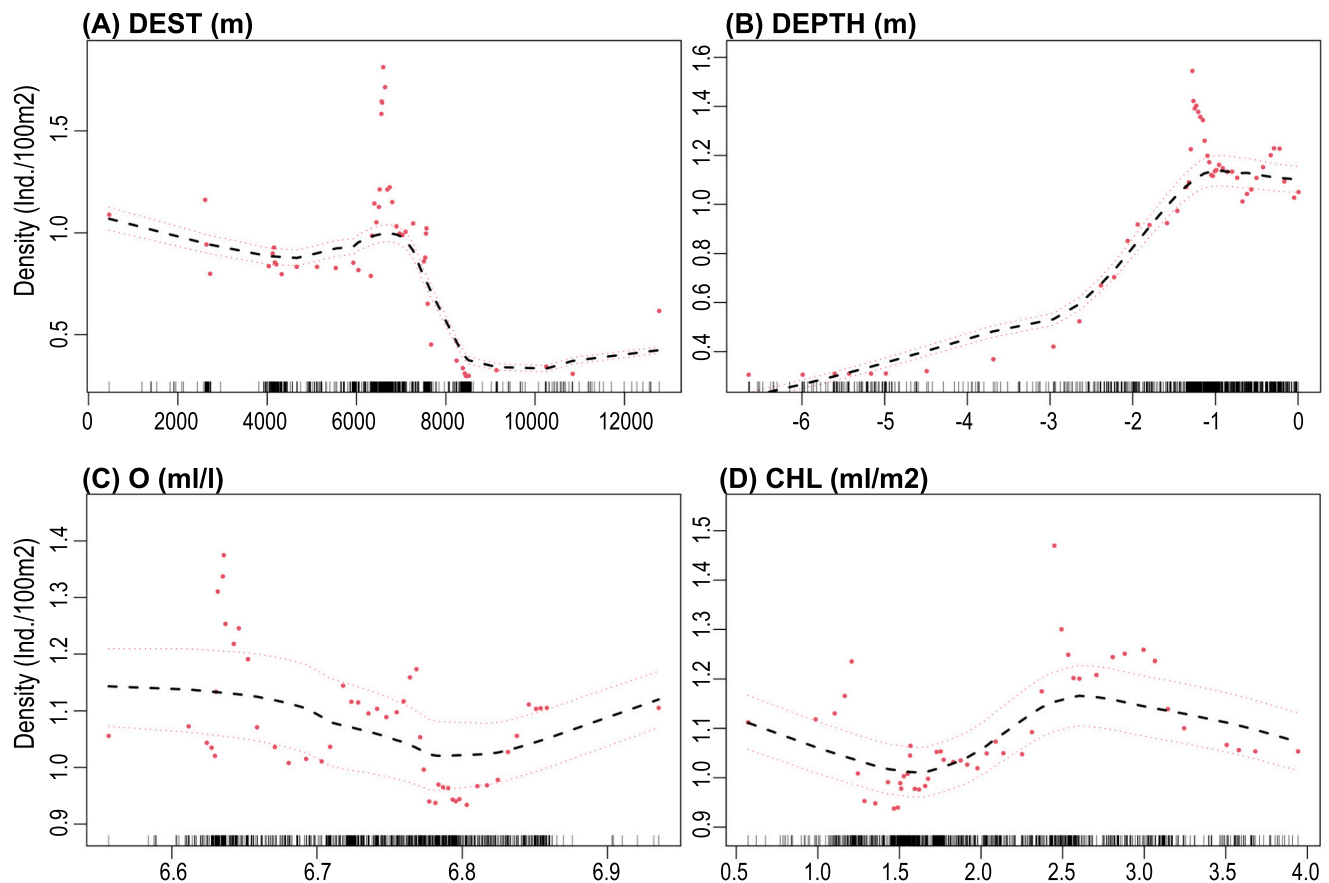


Fig. 9. (A–D) Effects of predictors in the Random Forest density model with the variables selected by *ffs*. The variables are ordered from highest to lowest importance in the model (Fig. 9(B)). The black dashed line represents the estimated effect, the dotted red lines the 95 % confidence intervals, and the red dots indicate partial values for observed points. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

This distribution pattern corroborates the depth limit marked by the hypoxia and anoxia episodes of the second half of 2015 and 2016 (Belando et al., 2017; Jiménez-Martínez et al., 2016). The environmental conditions associated to eutrophication process determine the current distribution of *P. nobilis* in the Mar Menor; no surviving specimens have been found below 3 m. depth, coinciding with the threshold of destruction of the benthic community after the eutrophication crisis (Belando et al., 2017; Ruiz et al., 2020). These results, which coincide with the data obtained in the present work, do not coincide with the results published in Nebot-Colomer et al. (2021), regarding the presence of live pen shell specimens in 2017, in zone zero of the 2016 eutrophication episode. This episode caused the death of >90 % of the existing population in 2014. The results obtained from the shells found demonstrate a significant change in the distribution of abundance by depth, compared to the current distribution of living specimens. Whereas the highest density of dead shells (many of which were still anchored in the sediment) was found between 5.6 and 6 m. depth, live specimens are

now restricted to areas above 3 m.

Likely, the eutrophication episode also affected a percentage of specimens above this threshold, as 17 % of shells from dead specimens were found there. The fragility of the shell makes it unlikely that whole specimens would be found after a certain period of time, so these data could be a rough indicator of how shallower areas were affected by this event.

The distances to the channels connecting to the Mediterranean do not show a gradient pattern for *Pinna nobilis* distribution. The probability of finding specimens in nearby areas is 30 %, much lower than that found in 2014 (55 %). It is striking that the distance at which this probability begins to increase coincides with the most important population locations in the lagoon: *Pueblo Cálido* (2750 m. DEST), *Cabo Romano* (6055 m. DEST), *El Barón* island (6580 m. DEST) and *La Perdiguera* island (7200 m. DEST). This pattern with a maximum at 10,000 m distance, was not detected in 2014. The observed presence probability near *El Estacio* is approximately 35 %, which contrasts with the values

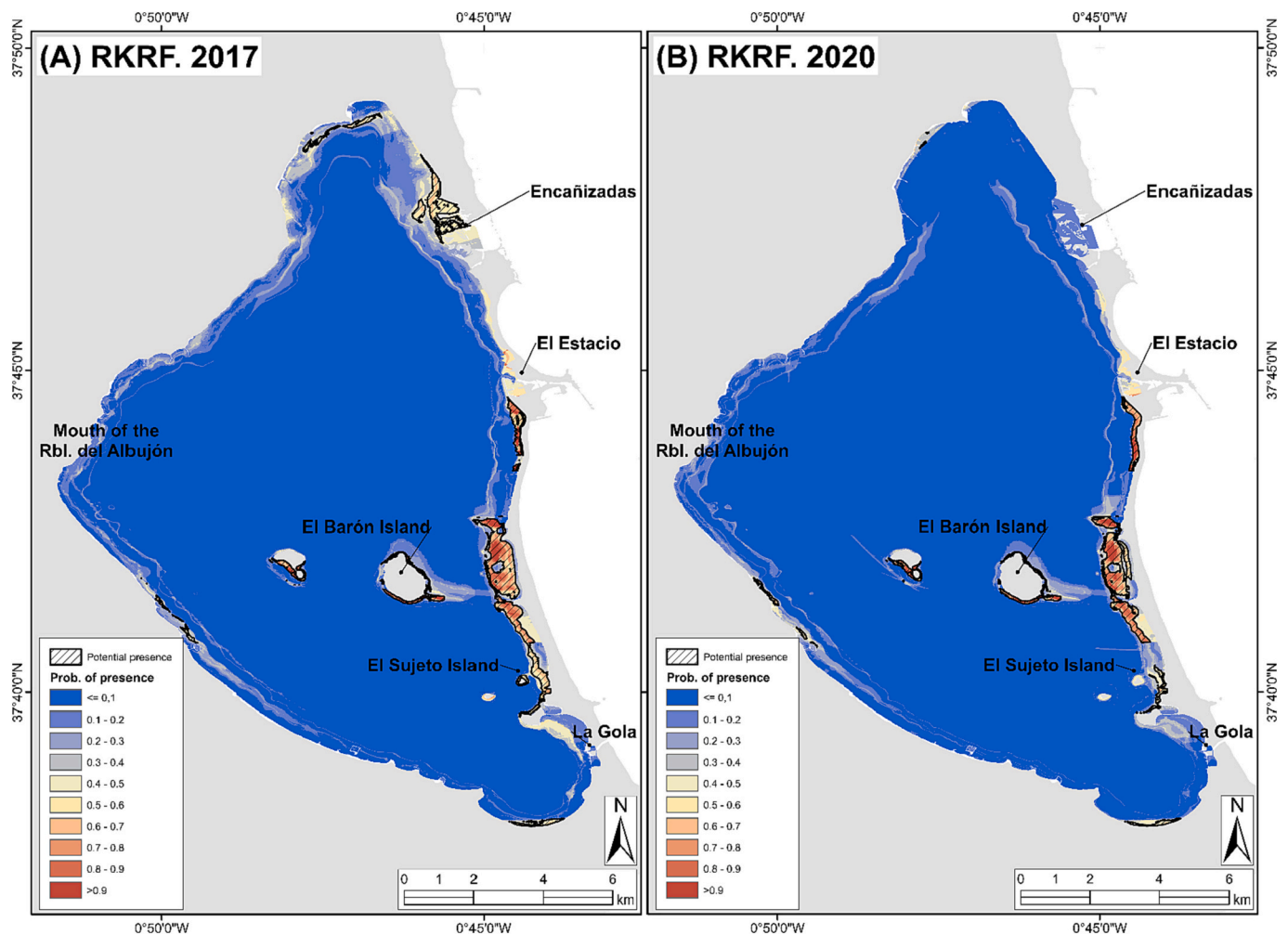


Fig. 10. Potential distribution of *P. nobilis* in (A) 2017 and (B) 2020. Presence-absence for RKRF models with variable selection (hatched areas refer to potential areas of distribution based on probability of occurrence above the cutoff point).

above 50 % detected in 2014, but while in 2014 probability began to decrease beyond 5000 m, the present study detects an increase in probability from 2500 m (approximate distance to the surviving population of *Pueblo Cálido*) to a maximum at around 6500 m, with two plateaus coinciding with the distance to the populations of *La Perdiguera* island, *Cabo Romano* and *El Barón* island.

The *P. nobilis* sub-populations in areas above 3 m remained stable, with very low mortality rates. However, in the area of influence of *las Encañizadas*, the northern basin, an abundant number of specimens was observed at the beginning of 2017 (Giménez-Casaldueiro et al., 2020) that suffered a significant regression during the following months, ending with the death of all the sub-populations in 2018, probably due to Haplosporidian infection.

The period from October 2016 to January 2017 was the wettest in the region in the last 75 years (AEMET, 2022). The abundant rainfall around the Mar Menor caused a drop in salinity even greater than during the 2019 event (36.13 psu on 5 April 2017) (Fig. 12).

This drop in salinity coincides with the possible input of Mediterranean water from *Las Encañizadas* and *El Estacio* channels produced by different storms in January and February 2017 with a swell up to 6 m at the *Cabo de Palos* buoy (MITMA, 2022) offering an opportunity for the entry of *Haplosporidium pinnae* from the Mediterranean, this explains the disappearance of *P. nobilis* near the entrances on those dates, as observed in the census of the present study. However, the possible entry of the protozoan was apparently controlled by the rapid increase in salinity of the lagoon, which reached 42 psu in less than three months (UPCT,

2022), values that should reduce the viability of the pathogen (36.5–39.7) and prevent its expansion (Cabanelas-Reboredo et al., 2019). This idea also sheds some light on the infestation data of some specimens by *H. pinnae* in 2019, in areas further away from *Encañizadas* and *Estacio*, southwards from the lagoon found in Nebot-Colomer et al. (2021).

Moreover, in the second half of 2017 an extraordinary growth of serpulids of the genus *Hydroides* (*H. dianthus* and *H. elegans*) was detected as a result of the availability of suspended organic matter and bacterioplankton (Sandonnini et al., 2021a, 2021b). Its rapid growth on some *P. nobilis* specimens was the cause of a new episode of mortality because it blocks their shells and collapses their filtration capacity. Other authors have also detected sub-lethal effects (slower growth) in some *P. nobilis* specimens due to invasive algae growing on their shells (Kersting and García-March, 2017).

Between January and September of 2018, the general environmental conditions in the Mar Menor improved due to policies controlling agricultural discharges and favourable weather conditions with very low precipitation (AEMET, 2022). However, between September and November 2018, several rainfall events affecting the Mar Menor produced new nutrient inputs coinciding with the winter water temperature drop, so it had not immediate eutrophication consequences, although could have a cumulative effect.

The first half of 2019 was dry with the exception of April (AEMET, 2022). A beginning of eutrophication was detected in the southern basin during the July sampling campaign, possibly due to the previous

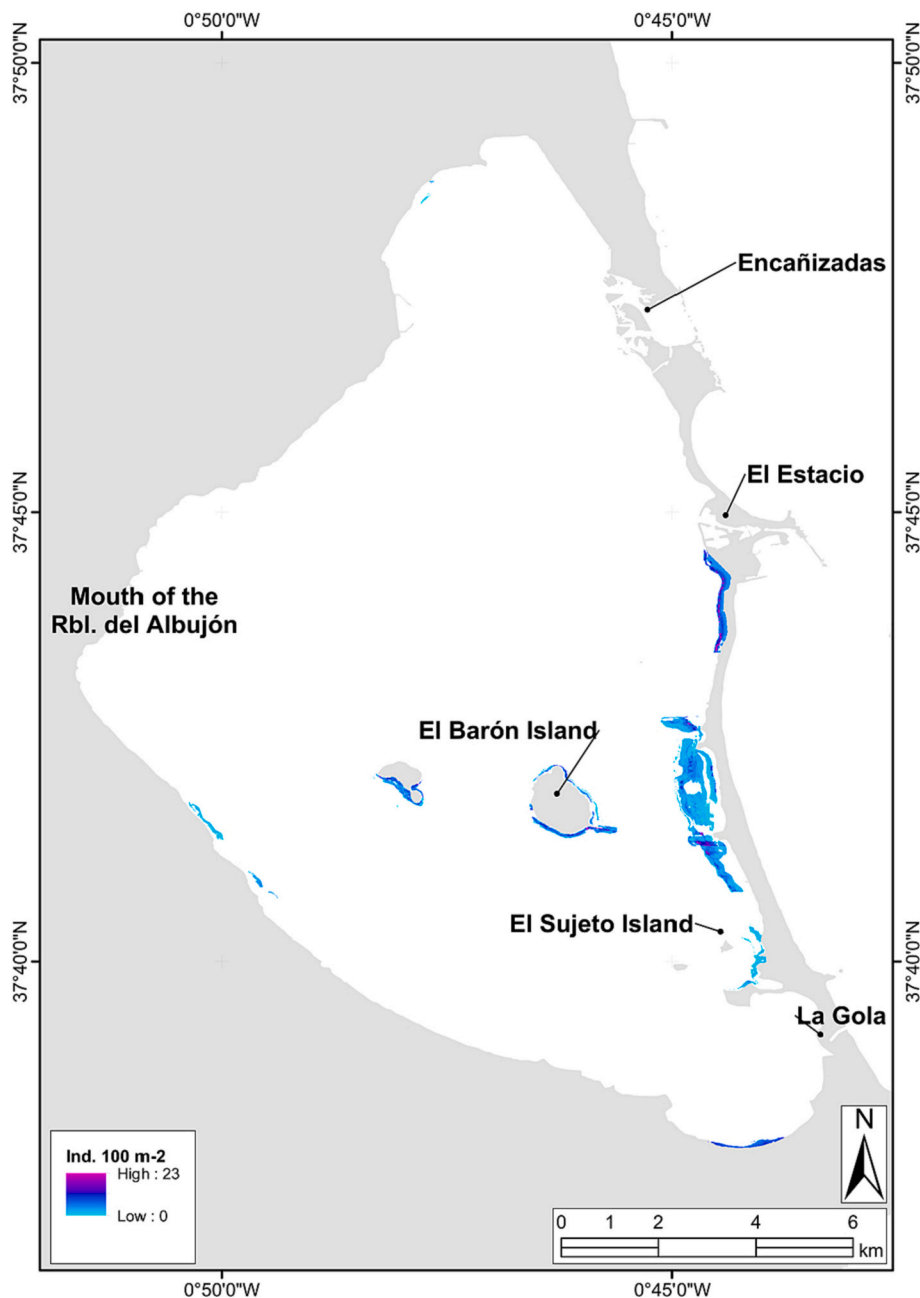


Fig. 11. Spatial prediction of density of *P. nobilis* in 2020: RFRK model with variable selection using potential areas from Fig. 9(A) as a mask.

accumulation of nutrients during the winter months. In August, abundant rainfall events produced nutrient inputs coinciding with maximum water temperatures and a eutrophication process already activated. In addition, a relaxation of discharge control measures was observed, which accelerated the eutrophication process.

On the 12th and 13th September 2019, a Cut Off Low (COL) affected the Mar Menor basin with rainfall amounts larger than 300 l/m^2 , registering the historical record of precipitation in 24 h (MITECO, 2019) that caused a 80 cm sea level rise with very negative consequences, as it coincided with the episode of eutrophication. This sea level rise generated outflows of water towards the Mediterranean through *las Encañizadas* and *el Estacio* for a period of around 20 days. The differences in salinity between the incoming fresh water and the lagoon water favoured the stratification of the water mass (Romero-Díaz and Pérez Morales, 2021). A shallow, low-salinity body of water was detected with a new process of eutrophication underway, which increased even more

with the input of nutrients carried by rainwater, and on the other hand, a deep, high-salinity body of water, where the decomposition of organic matter that had begun months earlier continued its course causing extreme situations of anoxia and generation of toxic compounds from the metabolism of the dominant anaerobic bacteria in that layer of water. Once the massive outflow of water between the Mar Menor and the Mediterranean was balanced, due to the anticyclonic situation, there was a period of time without practically any exchange of water between both water masses (Gilabert, J., pers. Comm.).

It is likely that the reinfection of individuals in different areas of the lagoon, observed in November 2019 (Nebot-Colomer et al., 2021), was due to the activation of resistance forms of the parasite, present in the Mar Menor since its first entry in 2017, during the new episode of low salinity.

In October 2019, a tilting movement of this anoxic and toxic mass, favoured by the prevailing winds, caused a new event of mass mortality

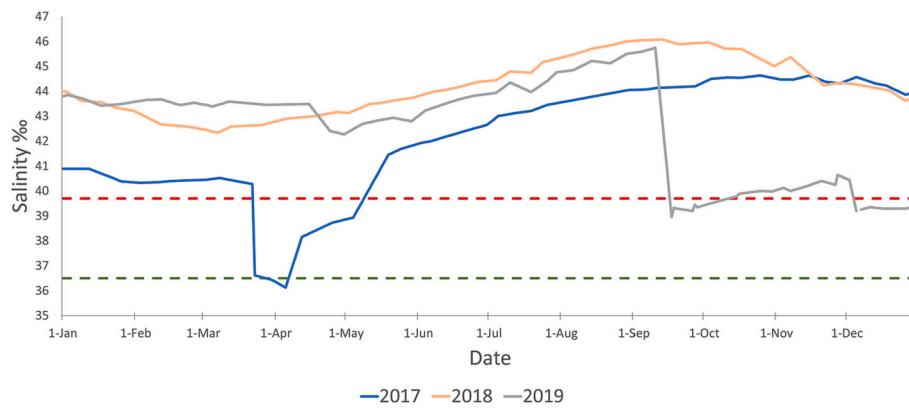


Fig. 12. Daily average salinity in the Mar Menor during 2017, 2018 and 2019. The dashed red line indicates the upper parasite infection threshold at 39.7 psu and the dashed green line indicates the lower parasite infection threshold at 36.5 psu. Data obtained from [UPCT \(2022\)](#). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of fish and invertebrates that affected the pen shell colony located in the “point zero” of this upwelling: *Playa de Villanitos* in *San Pedro del Pinatar*.

In August 2021, a new process of anoxia was detected in different areas of the bottom of the lagoon, again related to an excess of nutrients in the water and periods of eutrophication. On this occasion, a situation of prolonged hypoxia affected shallow areas close to *La Manga del Mar Menor*, causing a situation of stress in the colonies located in that area. The situation of the colonies is currently being evaluated, as a certain degree of mortality has been detected.

At present, the estimated population does not exceed 1500 specimens. The continuous instability of the system due to eutrophication, vandalism, illegal extraction of healthy specimens, and the intentional removal of specimens by some fishermen threaten the survival of the species. Suggested solutions, such as the dredging of the connection channels could decrease salinity by allowing the entry of Mediterranean water, which would favour the spread of the pathogen and the disappearance of the surviving specimens.

Regarding the use of SDM to characterise the spatial distribution of *P. nobilis*, [Katsanevakis and Thessalou-Legaki \(2009\)](#) estimate *P. nobilis* density in Souda Bay using a GAM model; [Rabaoui et al. \(2010\)](#) did the same using GLM and GAM models in Tunisia, and compared their results with other works in the Mediterranean. In Spain, [Vázquez-Luis et al. \(2014\)](#) use regression kriging to predict potential distribution in the Cabrera National Park and [Giménez-Casaldueiro et al. \(2020\)](#) use a two-stage RFRK to characterise *P. nobilis* temporal evolution and spatial distribution in 2003 and 2016 in the Mar Menor lagoon. We have seen in this study that goodness-of-fit statistics obtained by RFRK indicate higher accuracy than those of GLM and GAM models; so, RFRK is a good alternative to the SDMs methods normally used. The better results can be attributed to its higher flexibility to fit highly non-linear relationships ([Figs. 8 and 9](#)) and to the incorporation of spatial autocorrelation, as opposed to purely global methods, which can improve prediction in situations where the spatial distribution of the species shows a high concentration in localised areas.

Different actions, both “in situ” and “ex situ”, have been designed in order to maintain the viability of the pen shell population in the Mar Menor and achieve its recovery. The “in situ” actions include the monitoring of the current population, the surveillance against specific critical events that may affect specific areas of its distribution, the characterization of suitable areas for its survival where endangered specimens could be translocated, as well as the monitoring of reproductive events and the recruitment of juveniles using larval collectors.

The detection of successive annual reproductive events together with the observation of the viability of gametes and different larval stages (Cortés-Melendreras, E., pers. Obs.) indicates that there may be effective recruitment. Although no larvae have been obtained in the larval

collectors, juveniles have been observed in different areas of the lagoon. Deterioration of environmental quality directly affects reproductive effectiveness. On the one hand, the viability of larvae is limited in an aquatic environment with high bacterial concentrations ([Helm et al., 2006](#)) as a result of eutrophication and anoxia processes. On the other hand, when the reproductive event is successful and the juveniles settle, they are often affected by predation, mainly by gastropods such as *Hexaplex trunculus*.

Among the “ex situ” actions that could be considered more effective for the recovery of the species, not only in the Mar Menor, but throughout its distribution area, the establishment of protocols for maintenance and reproduction in captivity stand out.

All these actions aimed at the recovery of the species do not make sense if they are not accompanied by a set of effective measures aimed at improving the environmental conditions of the lagoon, which nowadays remain unstable.

Recovering the pristine Mar Menor of the 50s and 60s seems to be an impossible task, but to reach the environmental state observed in the lagoon during the golden age of the pen shells in the 90s and the first decade of this century only requires that politics and common sense go hand in hand.

5. Conclusions

In this contribution, the spatial distribution of *P. nobilis* in the period 2017 and 2020 and the factors that determine its downturn and/or survival are studied. These results are decisive when identifying the survival hotspots of the species and the tolerance threshold of the different environmental factors from which the death of the specimens occurs in the areas that have survived the *Haplosporidium pinnae* pandemic. In addition, the framework used in this study, based on a two-stage RFRK model, has turned out to be very useful to characterise the spatial distribution of species as a supporting variable for land-use planning and management.

CRedit authorship contribution statement

Emilio Cortés-Melendreras: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition

Francisco Gomariz-Castillo: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration

Francisco Alonso-Sarría: Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original

Draft, Writing – Review & Editing, Visualization, Supervision
 Javier Jiménez: Investigation, Resources, Data Curation
 Javier Murcia: Investigation, Resources
 Rosa Canales: Investigation, Visualization
 Alfonso A. Ramos Esplá: Review & Editing, Supervision
 Carmen Barberá: Investigation, Review & Editing, Visualization
 Francisca Gimenez-Casalduero: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing – Review & Editing, Visualization, Supervision, Project administration, Funding acquisition

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2022.114376>.

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