Predicting coexistence and predominance patterns between the introduced Manila clam (*Ruditapes philippinarum*) and the European native clam (*Ruditapes decussatus*)

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Abstract

In several European estuaries, the introduced Manila clam (*Ruditapes philippinarum*) has become a widespread and predominating species supplanting the native carpet shell clam (*Ruditapes decussatus*) whereas in other estuaries such as the Bay of Santander (Gulf of Biscay) this pattern has not been detected. Using this estuary as a case study, the potential coexistence/predominance patterns between these two species were explored with the objective of providing insight into the capacity of expansion of *R. philippinarum*. Firstly, the Ecological Niche Factor Analysis (ENFA) was applied to determine the niches of both species, using seven contemporary environmental variables, i.e. salinity, water depth, current velocity, and sediment sand, gravel, silt and organic matter content. Secondly, ENFA-derived habitat-suitability (HS) maps were simultaneously treated, using geospatial techniques and following HS index-based criteria, to determine the potential distribution patterns. Both species models performed well according to the cross-validation evaluation method. The environmental variables that most determined the presence of both clams were depth, current velocity and salinity. ENFA factors showed that *R. philippinarum* habitat differs more from the mean environmental conditions over the estuary (i.e. higher marginality) and has less narrow requirements (i.e. lower specialization). *R. philippinarum* dominated areas, determined by relatively lower current velocities and percentages of sand, higher organic matter contents and slightly shallower depths, were very reduced (i.e. 2.0 % of the bay surface) compared to

coexistence (47 %) and *R. decussatus* predominance areas (7.4 %). These results suggest that HS may regulate the expansion of *R. philippinarum*. ENFA, together with geospatial analysis of HS index, seems to be a valuable approach to explore the expansion potential of estuarine invasive or introduced species and thus support conservation decisions regarding native species.

Keywords [to be added]

1. Introduction

The natural or accidental invasions of marine non-indigenous species together with deliberate introductions can have significant impacts on native communities (Carlton, 1996; Ruiz et al., 1999). As rates of marine invasions continue to increase (Cohen and Carlton 1998), pressure will tend to increase on managers to minimize the potential impacts of non-indigenous species, and on scientists to provide control measures (Bax et al. 2001). However, management decisions for the conservation of a native species or actions against a certain introduced or invasive species are often hindered by a lack of basic and essential ecological information. Therefore, the study of the potential distribution patterns of native and non-indigenous species is essential to identify priority areas for conservation and to establish specific zone-based management plans. This information is even more important when the studied species are commercial and a sustainable fisheries management is desired. Estimation of distributions of these species can be linked with the potential yield of the fishery (Vincenzi et al., 2006a,b) and thus zone-based management plans may establish different fishing strategies.

The Manila clam (*Ruditapes philippinarum)*, native to the western Pacific Ocean (Scarlato, 1981), is one of the most world-widely introduced species for aquaculture purposes because of its high adaptability to various coastal environments and its suitability for aquaculture (Laing and Child, 1996; Usero et al., 1997). In several disturbed estuaries or lagoons of Europe such as Arcachon Bay (France) or the Lagoon of Venice (Italy), this species has supplanted the European native carpet shell clam (*Ruditapes decussatus*) by occupying similar ecological niches throughout its full range (Blanchet et al., 2004; Dang et al., 2010;

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Mistri, 2004). In the case of the Lagoon of Venice, it holds very high densities and has spread along the Adriatic coast at a rate of 30 km per year (Berber 2002). This extreme domination pattern has not yet been detected in other estuaries, such as the Bay of Santander (Gulf of Biscay, N Spain) (Juanes et al., 2012). However, being aware of the large-scale decline of *R. decussatus* in several estuaries of Europe, to explore the potential expansion of *R. philippinarum* seems to be essential for the conservation of *R. decussatus*. The conservation of *R. decussatus* is ecologically relevant in terms of concern over the consequences of biodiversity loss on ecosystem processes and ecosystem function, which subsequently affect the provision of ecosystem goods and services, and ultimately affect human well-being (Diaz et al., 2006). Moreover, specific zone-based strategies towards the sustainable fishery of the native clam are particularly crucial, since it is a much slower growing species and less resistant to unfavourable environmental conditions than *R. philippinarum* (Berber, 1985:1991; Usero et al., 1997).

The application of predictive models to obtain the potential distribution of marine species has increased, covering areas such as aquaculture (Longdill et al., 2008), fisheries management (Galparsoro et al., 2009), habitat management (Valle et al., 2011, Vasconcelos et al., 2013; Rinne et al., 2014) and conservation of a wide range of species such as cetaceans (Praca et al., 2009), migratory birds and turtles (Tian et al., 2008), polychaetes (Willems et al., 2008) and corals (Tittensor et al., 2009). With regards to bivalve species, habitat suitability predictions have been mainly focused on commercial species such as oysters and clams in order to improve management models or to restore habitats for aquaculture purposes (Soniat and Brody, 1988, Vincenzi et al., 2006a,b). Moreover, these techniques have also been used to predict spatial patterns of biological invasions and to prioritize locations for early detection and control of outbreaks of non-indigenous species (e.g. Inglis et al., 2006). Thus, these tools are now sufficiently mature to take on a larger role supporting conservation decisions, although linkage between research results and practice is still thin (Guisan et al., 2013).

Selecting the adequate modelling technique for the available data and objective of the study is critical to be as accurate as possible when predicting the potential distributions (Elith et al., 2006). Most of the available methods are based on presence/absence data (i.e. GLM, GAM, classification and regression tree analyses, and artificial neural networks). Brotons et al. (2004) suggest that GLM predictions (e.g. Vasconcelos et al., 2013) are more accurate than those obtained with the presence-only methods, such as Ecological Niche Factor Analysis (ENFA) (Hirzel et al., 2002), when species were using available habitats proportionally to their suitability, making absence data reliable and useful to enhance model calibration. However, absence data could be often of limited use because certain areas within the study site may be suitable, but has not yet been colonized by the invasive or introduced species (Hirzel et al., 2002). This suggest that pure presence-only methods are more likely to predict potential distributions that more closely resemble the fundamental niche of the species, whereas presence-absence modelling is more likely to reflect the present natural distribution derived from realized niche (Zaniewski et al., 2002). Therefore, ENFA may be particularly suitable when the objective is to predict the potential expansion of an introduced species and not the current status of colonization. This approach has proven to be a valuable tool for predicting habitat suitability of marine benthic species (e.g. Willems et al., 2008; Galparsoro et al., 2009; Valle et al., 2011) and for monitoring the potential spread of invasive or introduced species in terrestrial habitats (e.g. Hirzel et al., 2004a).

Within this context, ENFA was applied for *R. decussatus* and *R. philippinarum* in the Bay of Santander (N Spain, Gulf of Biscay) in order to (1) examine the influence of several ecologically relevant environmental variables in determining suitable habitats for both species and (2) explore potential coexistence and predominance patterns. The development of these objectives allowed testing the hypothesis that habitat suitability can play an important role in regulating the expansion and predominance of *R. philippinarum* over *R. decussatus*. To test this hypothesis, the following research questions concerning the distribution patterns between both species were posed: (1) is the habitat of the estuary more suitable for *R.*

philippinarum than for *R. decussatus?* and (2) is *R. philippinarum* predominance area larger than that where *R. decussatus* predominates? Previous data indicate that *R. philippinarum* is abundant and widespread in this estuary but a clear predominance pattern over the native clam has not been detected yet. Therefore, a priori, it was expected that habitat suitability might be regulating its expansion. The method applied and results of this study are intended to serve as a tool for conservation and ecosystem management dealing with introduced or invasive species.

2. Material and methods

2.1. Study Area

The study area is located in the Bay of Santander (Gulf of Biscay) (Figure 1), the largest estuary in northern Spain with an area of 22.7 km^2. The intertidal zone represents 67% of the total area of the bay (1573 ha) and is mainly concentrated in the right margin of the bay. Shellfishing of *R. decussatus* and *R. philippinarum* is conducted in seven intertidal fishing zones (Figure 1), using traditional techniques (i.e. hand rakes, knifes) (Juanes et al., 2012). A small *R. philippinarurm* farming area (~1 ha) is located in the tidal flat of Elechas (Figure 1). Galván et al. (2010) classified this estuary as morphologically complex and dominated by intertidal areas and tidal dynamics. The substratum of this area varies from sandy (northern open areas) to muddy (southern and inner areas) (Figure 2). Subtidal zones are dominated by shallow waters (< 5m), with maximum depths of 10–12 m along the navigation channel (Juanes et al., 2012). Hydrodynamic conditions are controlled by a semidiurnal tidal regime and a 3 m mean tidal range, interacting with variable freshwater inputs coming mainly from the river Miera through the Cubas area with a mean flow of 8 m^3 s^{-1} (Galván et al., 2010). Further details of this estuary and its sand flats are provided elsewhere (e.g. Puente et al., 2002, Juanes et al., 2012, López et al., 2013).

(Figure 1)

2.2. Clams presence data

Sampling surveys were conducted between April and May of 2010 to detect the presence of *R. decussatus* and *R. philippinarum*. Clams were collected at 39 stations (1m x10m transects) by a professional shell-

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woman by hand raking of the sediment, following Juanes et al. (2012). Transects were located in intertidal and shallow subtidal zones, considering these species depth range (Vincenzi et al. 2006a,b; Albentosa and Moyano, 2009). Locations where presence was documented were marked with a GPS device. Taxonomic determination of individuals was carried out in the laboratory. Both species presence maps (Figure 1) were transformed to Boolean raster-based grid files with identical characteristics to the environmental variables grids (see section 2.3) as required by the software Biomapper 4.0 for the ENFA computations (Hirzel et al., 2004b). Data were processed using ArcGIS 9.2.

2.3. Environmental variables

Different topographic (depth), physical (salinity, current velocity and sediment characteristics such as percentage of sand, gravel and silt) and chemical environmental variables (organic matter content in sediment) were obtained from field surveys and circulation models (Table 1). The studied environmental variables were selected for being meaningful to the ecology and distribution of these species (Laing and Child, 1996; Delalli et al., 2004; Vincenzi et al., 2006a,b; Cilenti et al., 2011). . The variable raster-based grids (244 x 298 cells of 51m x 51m) were obtained using the Kriging interpolation method in ArcGIS 9.2. The grid characteristics of the environmental variables were identical to the bathymetric grid, which was the limiting variable (i.e. depth) regarding resolution.

(Table 1)

2.4. Description of habitat

The distribution of the variables in (1) sites where species were present and (2) the distribution in the global area were compared in order to have an improved interpretation of each variable throughout the marginality factor obtained in the ENFA model. For this purpose, minimum, maximum and mean values and standard deviation were calculated for each variable in both species presence cells, as well as in the whole study area (Table 2).

2.5. Ecological Niche Factor Analysis (ENFA)

ENFA method was selected to analyze the potential distribution of the studied species. As a presence-only method, it may be particularly suitable over presence-absence methods when the objective is to predict the potential expansion of an introduced species that may not colonize the entire niche yet, since for this purpose absence data may be inadequate (Hirzel et al., 2002; Zaniewski et al., 2002). Moreover, with respect to assessing the importance of each variable, ENFA results are more straightforward to interpret than other presence-only models such as Maxent (Phillips et al., 2006) based on heuristic and jackknifing estimates outputs (e.g. Tittensor et al., 2009; Rinne et al., 2014).

ENFA compares distributions of eco-geographical variables between the locations where the species is present (species mean) and those in the whole area (global mean), thereby extracting the range of environmental conditions that the species inhabits (niche width) (Hirzel et al., 2002). Based on the niche width, ENFA calculates the habitat suitability indices of a species in relation to eco-geographical variables.

Model application and factors

ENFA was fitted in the software Biomapper 4.0, which implements all the procedures required to conduct the analysis (Hirzel et al., 2004). First, the Box-Cox algorithm was used to normalize the environmental variables (Sokal and Rohlf, 1995). Secondly, a covariance matrix was calculated in order to determine and remove from the analysis those variables that were highly correlated. ENFA was applied to both clam species, using previously obtained presence and environmental variables maps. The resulting factors that most explained the variance of the distribution were retained for the habitat suitability maps computation.

The first factor obtained is defined as the "marginality factor" (M), and the coefficients of M express the marginality of the focal species on each variable (see Table 3). A marginality value near to or greater than 1 indicates that a species lives in a very specific or marginal habitat in relation to the reference set (Hirzel et al., 2002). The remaining factors explaining the species distribution are defined as specialization factors (S) which indicate how restricted the niche of the species is in relation to the study area. The higher the

absolute value, the more restricted is the range of the focal species with respect to the corresponding variable. S ranges from 1 to ∞, with any value greater than 1 indicating a degree of specialization, with the niche becoming narrower as S increases. The tolerance (T) is the inverse of specialization and ranges from 0 to 1, with values closer to 1 indicating a wider niche (Hirzel et al., 2002).

Habitat suitability maps

The habitat suitability (HS) maps were computed calculating a suitability index for each cell of the raster-based grid, using the mean geometric algorithm. Each cell of the HS map was indexed with a value within the 0-100 range, where higher values indicate a more suitable habitat for the respective species (Hirzel et al., 2006). The predicted maps were reclassified into 4 HS index (HSI) classes using GIS techniques: unsuitable (HSI <25), barely suitable (25≤ HSI >50), moderately suitable (50≤ HSI ≤75), highly suitable (HSI >75). The number of cells was counted for each HSI class and the corresponding area was thus calculated.

Model evaluation

The predictive capability of the HS model was evaluated for both species, using the Jack-knife area-adjusted frequency Cross-Validation procedure (Hastie et al., 2001), following the method described by Boyce et al. (2002). Accordingly, the presence points of the species were divided into five groups. The data of four of the partitions were used to compute a HS model and the left-out partition was used to validate it on independent data. The Boyce index ranges from 0 to 1, and indicates the predicted accuracy of the HS model (Sattler et al., 2007) where values close to 1 indicate a higher predictive capability of the model.

2.6. Coexistence and dominance patterns

The areas where (1) one of the species was predominant over the other or (2), coexistence of both species, without specific significant predominance, occurs were determined using GIS techniques. For this purpose, the habitat suitability raster maps obtained were treated simultaneously using the raster calculator tool (ArcGis 9.2). A coexistence-predominance pattern map was obtained as follows: (1) predominance pattern was assumed in a cell if the difference in the HSI value between species was ≥ 50 and (2) coexistence

pattern was assumed if the HSI > 25 for both species and the difference between them was < 50. The areas corresponding to predominance or coexistence patterns and the distribution of the environmental variables values (mean ± SD) in these areas were calculated using the ArcGis 9.2 zonal statistics tool.

3. Results

3.1. Clam presence and habitat description

R. decussatus presence was recorded in 27 of the 39 stations, while *R. philippinarum* presence was recorded in 26 stations (Figure 1). Presence of *R. decussatus* with absence of *R. philippinarum* was found in the eastern tidal flats of Pedreña and Elechas and in the southern inner zones of Astillero, Solia and Tijero. The opposite trend was observed in the northeastern Cubas tidal fresh and in several central southern sand flats, between the port and Astillero.

R. decussatus presence was found in (1) depths between 0.6 m and 1.50 m below the MSL, which were shallower than the global mean, (2) areas with appreciable mean current velocities, slightly higher than the mean observed in the study area (0.29 m s^{-1} ±0.09), (3) particularly in sandy sediments but also in muddy ones (i.e. sediment at presence locations showed a high mean sand content (67.6%) and a high variance (SD= 31.50)), (4) water salinity values ranging between 26.0 and 34.1 with a mean similar to the global mean (31.5) and (5) sediments with a relatively higher organic matter content than the global mean (Table 2, Figure 2). While, *R. philippinarum* presence was found in (1) depths between 0.5 m and 1.50 m below the MSL, shallower than the estuary mean and similar to *R. decussatus*, (2) areas with lower mean current velocities (0.20 m s^{-1} ±0.10) than the global mean, (3) sediments with high percentage of sand (65.2% ± 30.80), (4) waters with a mean salinity of 31.4 (as for *R. decussatus*) values ranged between 26.0 and 34.1 and (5), sediments with a mean organic matter content higher than the global mean (see Table 2 and Figures 1-2).

(Table 2) (Figure 2)

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Regarding clam presence, significant differences between species were only detected for current velocities. *R. phillippinarum* was found in more sheltered zones (i.e. lower current velocities, Mann-Whitney U-test: U=200.0, Z=2.7, p=0.007) than the *R. decussatus*. The other variables did not show significant differences between species. These results suggest that, regarding the whole set of the presence locations, these species environmental preferences are similar except for current velocities. However, regarding predominance areas environmental conditions where significantly different between species as shown in section 3.4.

3.2. Ecological Niche Factor Analysis

First, the variables denoting the percentage of silt and gravel were removed since they were highly correlated (r >0.8) with organic matter and sand content, respectively. Then, 5 environmental variables were used to compute the ENFA, i.e. depth, current velocity, salinity, sand percentage, and sediment organic matter content.

R. decussatus

The overall marginality value of the ENFA analysis is 0.44, indicating that *R. decussatus* environmental requirements are different from the average environmental conditions of the Bay of Santander. A specialization value of 3.46 (>1) and a tolerance value of 0.29 indicate that this bivalve occupies considerably narrower ecological niches than *R. philippinarum*. Three factors (the marginality factor (M) and the two first specialization factors (S)) explain 98% of the distribution variance. The M factor explains 50% of the variability and the other two S factors explain the 41% and 7% of the variability, respectively (Table 3).

The environmental variables that most determine the presence of the *R. decussatus* (i.e. highest absolute value of coefficients along the M factor) are depth and current velocity, while the percentage of sand, salinity and sediment organic matter content are, in order of importance, relatively less explanatory (Table 3). Current velocity, percentages of sand and organic matter are positively associated with high habitat suitability (i.e., the mean values of these variables at presence locations are higher than those obtained for

the whole study area). Furthermore, depth and salinity are negatively associated with high habitat suitability (i.e. the mean values of these variables at locations where the species are present are lower than those obtained for the whole study area) (Table 2).

Salinity is of critical importance in explaining the specialization of this species (Table 3) as is shown by the higher values of the coefficient of this variable along the first S factor. In order of importance, sediment characteristics (high percentage of sand), depth (showing a narrow range of depth where the species was present) and current velocity play an appreciable role in explaining the specialization of the *R. decussatus*. Regarding the organic matter content of the sediment, it appears that this variable does not have a strong effect in determining the niche of these species.

(Table 3)

R. philippinarum

For the *R. philippinarum*, the overall marginality value is 0.65, which is slightly higher than that obtained for the native *R. decussatus*. This indicates that environmental conditions required by the introduced clam are also different from the mean environmental conditions of the study area (Table 2) and relatively different from the conditions required by the native clam.

The specialization (S) value is greater than 1 (2.12) and the tolerance value is 0.47, indicating that this species occupies narrow ecological niches, although wider than those inhabited by the *R. decussatus*. Four factors explained 95% of the variance of the distribution of this species. The M factor explains 63% of the variability. The other three S factors explain 18%, 7% and 7% of the variability, respectively (Table 3).

The environmental variables that most determine the presence of the *R. philippinarum* were depth and current velocity, whilst salinity and the percentage of sand are relatively less important (Table 3). The percentage of sand and organic matter content are both positively associated with high habitat suitability,

while depth, current velocity and salinity are negatively associated. Salinity is the key variable explaining the specialization of this species as is indicated by the higher values of its coefficient along the first S factor (Table 3). Sediment characteristics, depth and currents also play a role in explaining the specialization of the species (see factor 1 and 2 of specialization). However, the role of the organic matter content determining the habitat suitability for this species is not as important as that for the native clam.

3.3. Model evaluation and habitat suitability maps

R. decussatus model

Cross-validation of the quality of the model resulted in a Boyce index of 0.82 ± 0.10, indicative of the high predictive power of the model. The habitat suitability map shows that 20% of the surface (407 ha) of the Bay of Santander is highly suitable for *R. decussatus* and an additional 21% (440 ha) is moderately suitable (Figure 3a). These habitats (HS index >50) are mainly located along the northern and outer zones in Cubas and along the eastern Elechas and Astillero tidal flats, with a limited presence in the southern inner zones (Figure 3a, see also the original Figure S1a without reclassifying in HSI classes).

(Figure 3)

R. philippinarum model

The high Boyce index (0.77 ± 0.08), indicates that the model is a good predictor of the habitat suitability for *R. philippinarum*. The model shows that 16% of the surface of the bay (317 ha) is highly suitable for the development of this species. Moreover, another 16% of the bay corresponded to moderately suitable habitats. These habitats are located along the eastern tidal flats of Pedreña and Elechas and the southern inner zones (i.e Solia and Tijero tidal fresh), with a limited presence in the Cubas northern area (Figure 3b, see original Figure S1b without reclassifying in HIS classes). The most important differences in the distribution of suitable habitats between species are located in the north and central zones of the Bay, where conditions are more suitable for the *R. decussatus*, and in the southern areas, where the habitat is more suitable for the *R. philippinarum*.

3.4. Coexistence and predominance patterns

The potential coexistence of both clams is the most common distribution pattern, covering 974 ha (47.1% of the bay surface) mainly located in the eastern areas of the estuary. In contrast, potentially unsuitable conditions for both species are present in relatively more oceanic central and northeastern areas, but also in southern more estuarine inner zones, covering in 899 ha (43.5%) (Figure 4).

R. decussatus potentially predominates in a surface of 153 ha (7.4%) mainly located in the north and central-southern zones of Cubas and Elechas-Astillero, respectively. Here, environmental conditions are similar to those found where both species coexist (Table 4). The main difference between *R. decussatus* potential predominance areas and coexistence areas is regarding the current velocity, being stronger for the former. While, *R. philippinarum* potentially predominates in a surface of 42 ha (2.0%), distributed in the southern inner of Tijero and Solía, and a reduced area of Elechas. *R. philippinarum* potential predominance areas are characterized by relatively shallower waters, lower current velocities and percentage of sand, and higher sediment organic matter content with respect to coexistence and *R. decussatus* predominance areas.

(Figure 4) (Table 4)

4. Discussion

The ENFA presence-only model was first used to construct habitat suitability predictions for two congeneric bivalves, the European native carpet shell clam (*R. decussatus)* and the worldwide-introduced Manila clam (*R. philippinarum*), with the purpose of determining the potential coexistence and predominance patterns in the Bay of Santander (Gulf of Biscay). The results of this study provide consistent support for the initial hypothesis that habitat suitability may regulate the expansion of *R. philippinarum*. The answers to the questions raised concerning the distribution patterns between both species defend this hypothesis: The habitat of the estuary is potentially less suitable for *R. philippinarum* than for *R. decussatus,* and the predominance of *R. philippinarum* is a much less common pattern than coexistence of both species or predominance of *R. decussatus*. Similar results were expected since previous

surveys suggested that *R. philippinarum* was abundant and widespread in this estuary but a clear predominance pattern over the native clam has not yet been detected (Juanes et al., 2012).

ENFA results show a high predictive accuracy for both species, according to the accepted statistical test (Boyce et al., 2002). Despite the overall higher accuracy of presence/absence models with respect presence-only methods such as ENFA (Brotons et al., 2004) the fact that the realized niche of *R. philippinarum* might be yet narrower than the potential fundamental niche, makes the latter approach more adequate for this specific study (Hirzel et al., 2002; Zaniewski et al., 2002). Moreover, ENFA is particularly useful for comparing the relative distributions of these two clam species since the information come from a single data set and the survey coverage for both species was the same. Therefore, any differences in the distribution of these species are likely to relate to real differences between them (MacLeod et al., 2008). This is importantly useful when assessing two biologically different species (Berber, 1991; Usero et al., 1997) with different management/conservation needs.

The habitat of both species differs considerably from the average conditions of the Bay of Santander. *R. philippinarum* shows a higher marginality than *R. decussatus*, while it is less restrictive in selecting the range of conditions where it is distributed (i.e. lower specialization). This is consistent with its non-indigenous species condition, great success showed in colonizing estuaries all over the world, and the possibility that first introduced in the late 1980s in the Bay of Santander, it may have behaved as a specialist during the early stages of colonization, becoming more generalist as the population expanded (Hilden, 1965; Sol et al., 1997).

Extracting the environmental variables which best explain the distribution of a species using ENFA require previous basic analysis. Variables that were strongly correlated with high habitat suitability might not necessarily be drivers, as they may be merely correlated with other important variables. In the present

study, there was strong correlation between organic matter and silt content and also between gravel and sand content (r >0.8). This further complicates the understanding of the relative importance of each individual environmental variable (Tittensor et al., 2009). Nonetheless, the relative importance of each environmental variable in the ENFA model (Table 3) provides some guidance about the drivers and interactions of the two species and the overall natural system. Among the variables considered in this study, those that more importantly explain the variance of both species distribution are depth and current velocity, with respect to the marginality, and salinity and sand percentage, with respect to the specialization. Current velocities and organic matter also play an important role in determining the specialization of *R. decussatus* and *R. philippinarum,* respectively. Both species are known to occur primarily on intertidal or shallow subtidal areas and are often associated with absence of strong currents and muddy to sandy sediments (e.g. Laing and Child, 1996; Gosling, 2003; Vincenzi et al. 2006a,b; Albentosa and Moyano, 2009). The distribution of both species was similar in terms of depth (0.5 m to 1.5 m below MSL), which is associated with optimal conditions for growth of clams of the genus *Ruditapes* (Cilenti et al., 2011). Regarding current velocity, both species are filter feeders and need a certain minimum current speed to produce enough food circulation and resuspension in the areas they inhabit. However, *R. philippinarum* requires more sheltered areas than *R. decussatus* (i.e. negatively and positively associated to this variable regarding the marginality factor, respectively).

These observations were reproduced in the habitat suitability and coexistence/predominance maps obtained in the present study (Figures 3 and S1, Table 4). Highly suitable areas for *R. decussatus* are mainly located along the more oceanic or open northern and eastern tidal flats, while those for *R. philippinarum* are more importantly present along the eastern and southern inner and more estuarine zones. *R. philippinarum* predominated over *R. decussatus* in very restricted areas characterized by muddy tidal flats with a high content of organic matter susceptible to resuspension in each tidal cycle (Figures 2 and 4). These results are in agreement with the feeding habits of these species. Growth rate of *R. decussatus* is importantly related to the primary production of the water column (Bodoy and Plante-Cuny, 1984; Page and Lastra, 2003), while

R. philippinarum seems to mainly feed on particulated organic matter resuspended from the sediment (i.e. detritus and micorphytobenthos) (Watanabe, 2009), more abundant in inner sheltered muddy zones of the estuaries with high organic matter inputs (Junoy and Viéitez, 1990; Mendes et al., 2006).

The low variance of the Boyce index obtained for both species indicates that the models are highly reproducible. However, the estimation of the species potential distribution can be affected by the range of environmental conditions over which the model has been applied, which may be a subset of the full environmental niche (Braunisch et al., 2008 and references therein). This may reduce the model predictability in this estuary and transferability to locations with different habitat settings. For such objectives it is recommended to consider most of the species environmental gradient (Pearson et al., 2002) by means of a higher level of sampling effort regarding species presence and environmental data. Thus, the best possible approximation of the ecological niche would be obtained. Such effort could include sampling or model data regarding phytoplankton concentrations, food supply, dissolved organic matter availability or dissolved oxygen concentration (Vincenzi et al., 2006a; Cilenti et al., 2011). In addition, although similarities between the species regarding the habitat suitability and the importance of the variables determining this suitability might be attributable to the fact that they are congeneric species, the resolution of the grid (51 m x 51 m) could also be contributing to this. Therefore, further analysis with higher spatial resolutions as new bathymetric data become available seems essential to (i) detect many of the topographical features associated with clams and (ii) evaluate whether the structure of the model design is affecting the niche estimations.

The high habitat suitability, potential predominance areas and the underlying processes and interactions have potential implications for the understanding of the distribution of both species, and development of zone-based conservation and management strategies for estuarine or lagoon ecosystems. However, the results should be treated with some caution taking into account that the introduced species might not have

fully occupied its fundamental niche due to limiting factors present in its habitat (Hutchinson, 1957). The presence of competing species or predators in an environment is one example of a limiting factor that restrains, narrows, or constricts an organism's ecological niche (Rodríguez-Caval et al., 2012). With this hypothesis in mind, the results presented here may have underestimated both species fundamental niche, since a recent study conducted by Bidegain and Juanes (2013) in this estuary has demonstrated the large effect of predation on both species, particularly on *R. philippinarum*. The alien species shorter siphons and lower tendency to bury (Vilela, 1950; Lee, 1996; Gosling, 2003), in comparison to *R. decussatus,* seems to make it significantly higher vulnerable to predation. Thus, predation might be playing an important role driving the distribution of both species and be a critical factor in the regulation of the expansion of the *R. philippinarum*. In order to have a reliable spatial distribution of this factor integrated in the ENFA, a large-scale experimental study adapting, for instance, Bidegain and Juanes (2013) could be conducted covering different tidal flats and sea levels. To our knowledge the integration of predation in an ENFA analysis might be a very thought-provoking novel approach.

Apart from the suitability of the colonized area regarding environmental variables and the regulation due to predation, the number and intensity of introductions may be an important factor enhancing/regulating the expansion of *R. philippinarum*. This species forms dominant populations in Arcachon Bay and Lagoon of Venice, where they have been introduced intensively and repeatedly, in large farming areas (24 ha and 900 ha, respectively) (Mantovani et al., 2006; Toupoint et al., 2008). However, in the Bay of Santander, the introductions have been much less intensive and frequent (1 ha located in a unique site, Elechas) (see Figure 1). Therefore, the low intensity of introductions in this estuary, together with the habitat suitability and predation filters, seems to be important in preventing the *R. philippinarum* from duplicating the predominance patterns observed in other estuaries in Europe.

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With respect to the management and control of *R. philippinarum* potential expansion and the conservation of *R. decussatus*, a zone-based strategy focusing fishery efforts towards areas defined as 'coexistence' or 'predominance zones' might be an interesting approach. In addition, the predation risk and the population connectivity, as important constraints in the presence of species, should support farming sites selection and restoration strategies. The most suitable locations for *R. philippianarum* farming sites might be those with highly suitable habitat, where the larval dispersion after spawning does not lead to significant retention rates within the estuary (Bidegain et al., 2013). In contrast, for the restoration or cultivation of *R. decussatus*, the most suitable sites should be those where predation risk is low and spawning leads to high recruitment within the estuary, resulting in an increase of the population stock.

5. Conclusions

ENFA was successfully applied to predict suitable habitats for the introduced clam *R. philippinarum* and the European native clam *R. decussatus*, and to explore the potential coexistence/predominance patterns in the Bay of Santander (Gulf of Biscay). The results suggest that habitat suitability importantly regulates the stage of development of *R. philippinarum* limiting the passage from an establishment-widespread stage to becoming dominant. The vulnerability to predation and current introduction intensities may also be contributing to limit the expansion. ENFA, together with geospatial analysis of HS index, seems to be a promising approach to explore the expansion potential of estuarine invasive or introduced species and thus support conservation decisions regarding native species.

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Figure footnotes

Figure 1 - Study area for the application of ENFA: Bay of Santander (N Spain, Gulf of Biscay). Distribution of presence locations are presented for *R. decussatus* (black quadrates) and *R. philippinarum* (grey circles).

Figure 2 – Environmental variables spatial distribution: (a) Depth (m), (b) Current velocity (m s^{-1}), (c) Salinity (Practical Salinity Scale), (d) Sediment sand content (%), (e) Sediment organic matter content (%), (f) Sediment silt content (%). Note that silt content is not used in the final ENFA analysis but it is presented to show the strong correlation with organic matter.

Figure 3 - Habitat suitability map for *R. decussatus* (a) and *R. philippinarum* (b) obtained by a reclassification of the original HS map (see Figure S1, supplementary material) into 4 HS index (HSI) classes: unsuitable (HSI <25), barely suitable (25≤ HSI >50), moderately suitable (50≤ HSI ≤75), highly suitable (HSI >75).

Figure 4 -Potential predominance and coexistence patterns of *R. decussatus* and *R. philippinarum* in the Bay of Santander.

Supplementary material, figure footnotes

Figure S1 – Habitat suitability map for *R. decussatus* (a) and *R. philippinarum* (b) obtained by ENFA.

Variable	Source	Type of data
Bathymetry	Nautical chart IHM 940, DTM	Raster map, interpolated data (mean sea level) derived from bathymetry data and nautical charts.
Current velocity	This study	Raster map of averaged current fields from modelled data, using H2D-A2D2 model (Bárcena et al., 2011, 2012; García et al., 2010 a,b; López et al., 2013) following Bidegain et al. (2013)
Salinity	This study	
Sediment characteristics % of sand % of gravel % organic matter % of silt	This study	Raster map, interpolated data from 56 sampling stations using ArcGIS 9.2.

Table 1 - Environmental variables, sources and types of data used in the present study.

	Max	Min	Mean	SD
Study area				
Depth	16.30	0.00	4.70	3.91
Current velocity	1.10	0.01	0.25	0.08
Sand	99.60	11.45	64.00	23.60
Salinity	34.16	0.00	31.80	3.80
Organic matter	10.70	1.20	3.57	2.40
R.decussatus **presence locations**				
Depth	1.50	0.60	1.30	0.35
Current velocity	0.40	0.05	0.29	0.09
Sand	98.60	11.70	67.60	31.50
Salinity	34.09	26.00	31.50	2.45
Organic matter	10.68	1.12	4.95	3.27
*R. philippinarum pre*sence **locations**				
Depth	1.50	0.50	1.00	0.43
Current velocity	0.35	0.01	0.20	0.10
Sand	98.30	11.45	65.20	30.80
Salinity	34.10	26.00	31.40	2.35
Organic matter	10.68	1.12	4.78	3.16

Table 2 - Distribution of the values of the 5 environmental variables considered in ENFA analysis: Depth (m), current velocity (m s^{-1}), sediment sand content (%), salinity and sediment organic matter content (%). For each variable, maximum, minimum, mean value and standard deviation are presented for the whole study area and for the presence locations of *R. decussatus* and *R. philippinarum*.
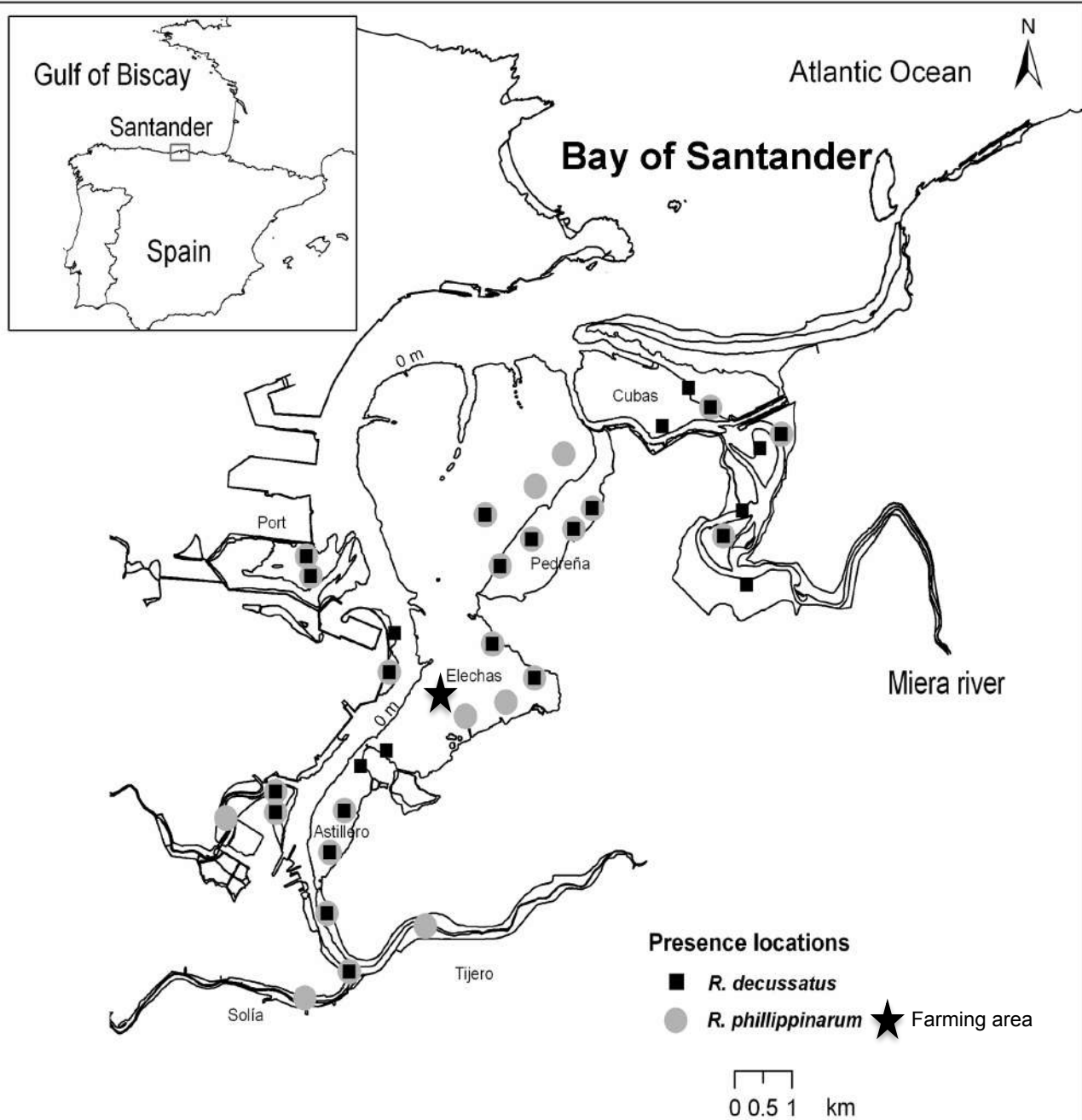
	R. decussatus			
Marginality (50 %)	**Specialization 1 (41 %)**	**Specialization 2 (7 %)**	**Specialization 3 (1 %)**	**Specialization 4 (1 %)**
Depth (-0.65)	Salinity (-0.71)	Depth (-0.61)	Organic matter(0.86)	Current velocity (-0.83)
Current velocity (0.45)	Sand (-0.56)	Salinity (-0.53)	Current velocity (-0.46)	Organic matter(-0.44)
Sand (0.30)	Depth (0.35)	Sand (0.41)	Salinity (0.17)	Sand (0.32)
Salinity (-0.26)	Current velocity (-0.24)	Current velocity (0.35)	Sand (0.12)	Salinity (-0.09)
Organic matter (0.16)	Organic matter (-0.05)	Organic matter (-0.24)	Depth (-0.09)	Depth (0.08)

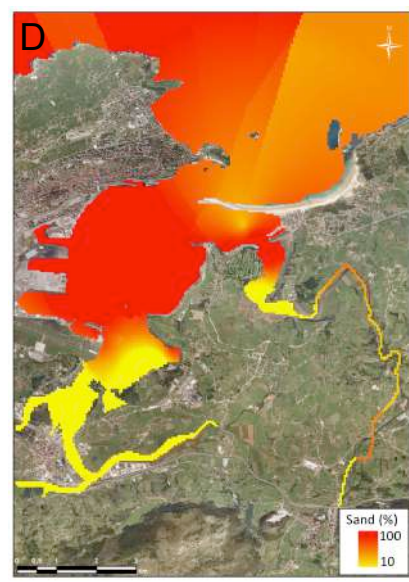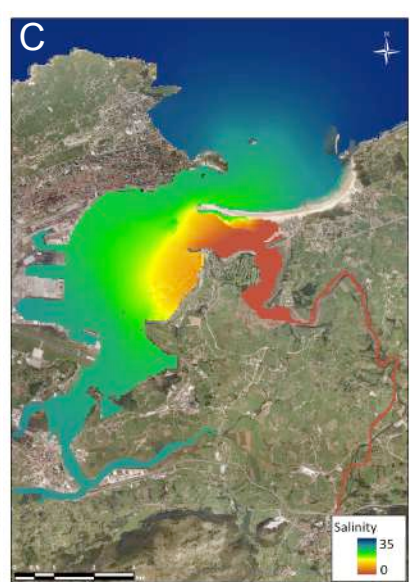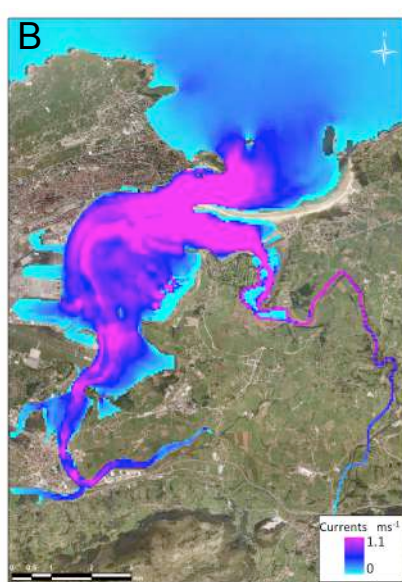|	***R. philippinarum***			
Marginality (63 %)	**Specialization 1 (18 %)**	**Specialization 2 (7 %)**	**Specialization 3 (7 %)**	**Specialization 4 (5%)**
Depth (-0.71)	Salinity (-0.89)	Depth (-0.69)	Sand (0.74)	Organic matter (0.94)
Current velocity (-0.58)	Sand (-0.35)	Current velocity (-0.68)	Organic matter (0.53)	Salinity (0.32)
Salinity (-0.30)	Depth (-0.22)	Salinity (-0.22)	Depth (-0.36)	Current velocity (-0.14)
Sand (0.28)	Organic matter (0.16)	Sand (0.12)	Salinity (-0.22)	Depth (0.03)
Organic matter (0.001)	Current velocity (0.02)	Organic matter (0.06)	Current velocity (0.02)	Sand (0.01)

Table 3. Variance explained (%) by five ecological factors (marginality and specialization factors) in the ENFA model for *R. decusatus* and *R. phillippinarum*. The environmental variables along the factors (depth (m), current velocity (m s^{-1}), sediment sand content (%), salinity and sediment organic matter content (%)) are listed in order of importance (absolute coefficient value) determining the marginality and specialization
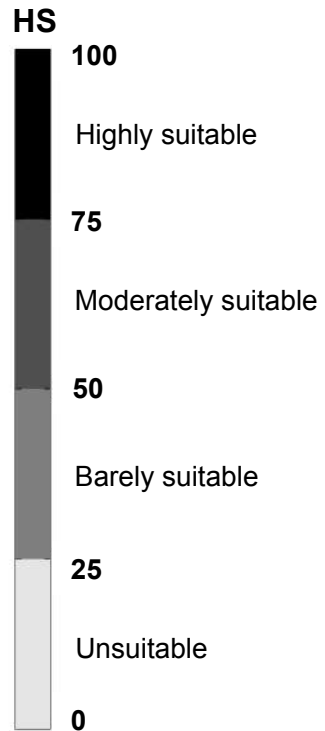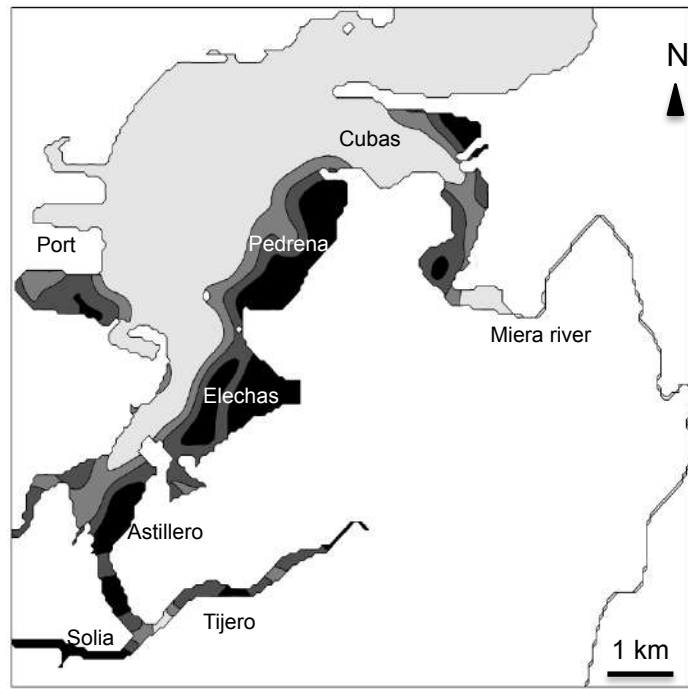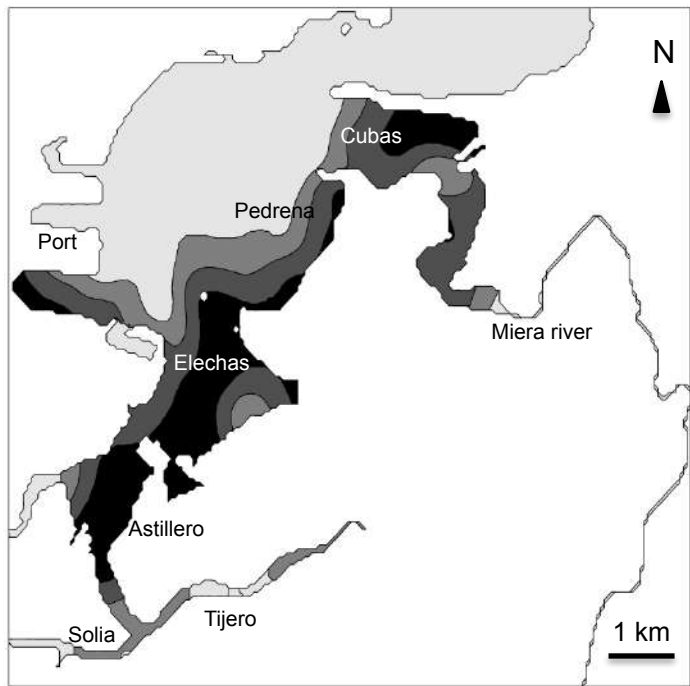
of the species. The sing of the coefficient values, along the marginality factor, indicates that the species prefers higher values (+) or lower values

(-) than the study area mean regarding the corresponding variable. The sign is arbitrary and has no interpretable meaning along specialization
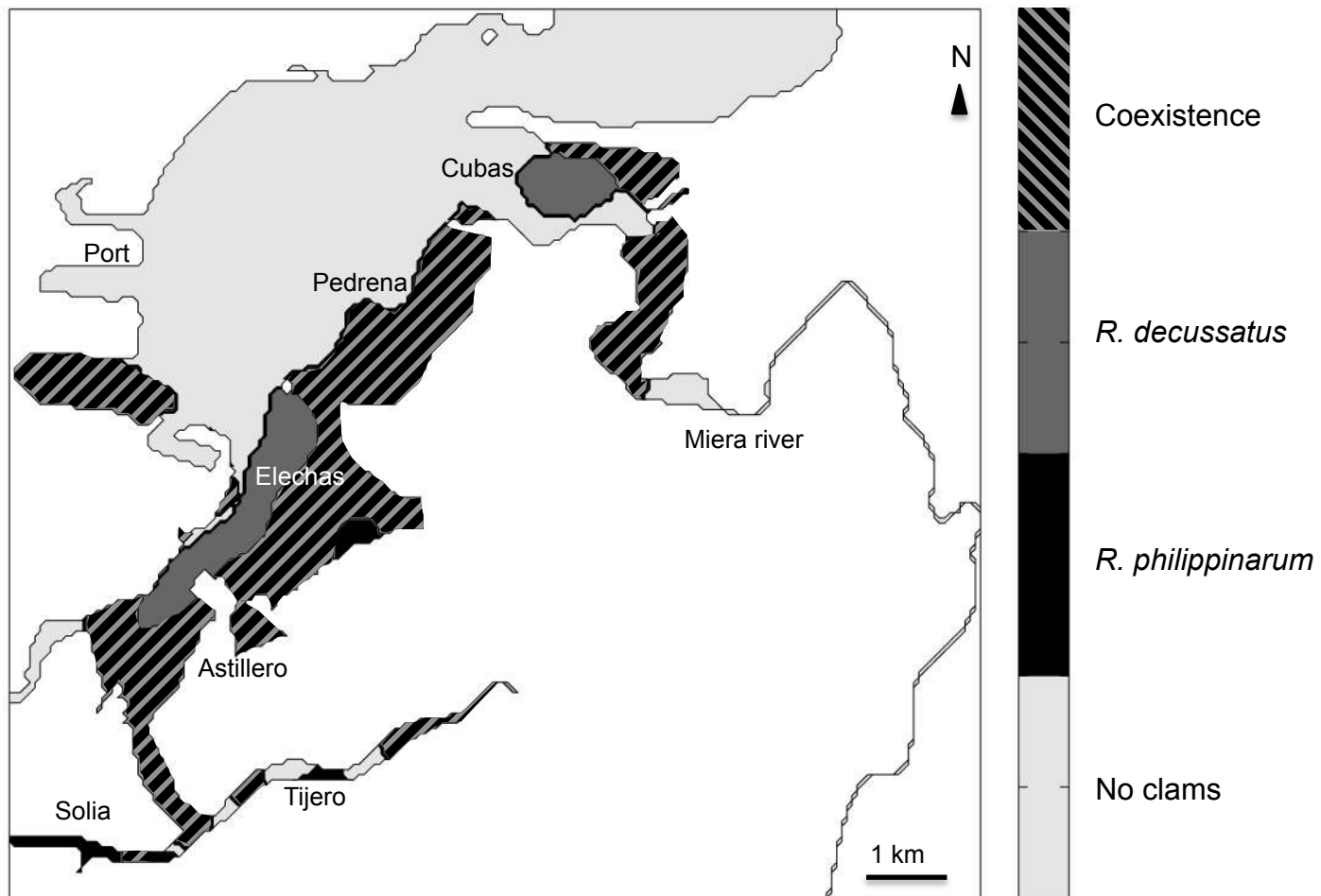
factors.

Variable	Coexistence	*R. decussatus* predominance	*R.philippinarum* predominance
Depth (m)	0.5 -1.5	1.1 -1.5	0.5 - 0.8
Current velocity (m s^{-1})	0.08 ($\pm$ 0.07)	0.18 ($\pm$ 0.05)	0.06 ($\pm$ 0.04)
Sand (%)	65.1 ($\pm$ 26.9)	65.6 ($\pm$ 17.8)	26.5 ($\pm$ 7.3)
Salinity	32.1 ($\pm$ 2.2)	32.2 ($\pm$ 1.7)	32.8 ($\pm$ 0.3)
Organic matter (%)	4.5 ($\pm$ 2.5)	4.7 ($\pm$ 1.1)	8.7 ($\pm$ 1.1)

Table 4 - Distribution of the values of the environmental variables used in ENFA for the areas where (i) both species potentially coexist, (ii) *R. decussatus* potentially predominates and (iii) *R. philipinarum* potentially predominates. For each variable the mean value ($\pm$ SD) or the minimum and maximum values (in the case of depth) are presented

Gulf of Biscay
Santander
Spain

Atlantic Ocean

N

Bay of Santander

Cubas

0 m

Port

Pedreña

Elechas

Miera river

0 m

Astillero

Tijero

Solía

Presence locations

■ *R. decussatus*

● *R. phillippinarum* ★ Farming area

0 0.5 1 km

Coexistence

R. decussatus

R. philippinarum

No clams

N

1 km

Cubas

Port

Pedrena

Elechas

Miera river

Astillero

Solia

Tijero

Highlights

- Coexistence of both species was the most common pattern.

- Predomination of *R. philippinarum* over *R. decussatus* was extraneous.

- Habitat-suitability limits the expansion of the introduced clam *R. philippinarum.*