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Molluscs collected with bottom otter trawl in the northern Alboran Sea: main assemblages, spatial distribution and environmental linkage

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Abstract

Molluscan assemblages of circalittoral and bathyal soft bottoms in the northern Alboran Sea were studied using an experimental bottom otter trawl. Samples of fauna were collected from 190 hauls over four MEDITS surveys carried out in spring between 2012 and 2015 at depths ranging from 30 to 800 m. Near-bottom measurements (temperature and salinity) and sediment samples were taken in the same locations as the faunal sampling. A total of 101 species grouped into 55 families were recorded. Cephalopods were the most abundant group, with *Abralia veranyi* dominating the abundance, *Octopus vulgaris* the biomass, and *Illex coindetii* and *Todarodes sagittatus* being the most frequently collected species. Multivariate analyses carried out separately with abundance data for demersal species, benthic species, bivalves, gastropods, and cephalopods, as well as total molluscs, generally resulted in three main molluscan assemblages corresponding to a shelf assemblage (30-200 m depth), an upper slope assemblage (201-350 m depth), and a middle slope assemblage (351-800 m depth). PERMANOVA analyses revealed that significant depth-related differences of distinct molluscs groups were more acute than for the geographical sectors of the Alboran Sea (western, central, eastern, and insular sectors). Abundance, biomass, and species richness all decreased with depth, with a clear dominance of cephalopods on the slope. Significant geographical differences were detected, principally for demersal and benthic species as well as gastropods and cephalopods, between the insular sector (Alboran Island) and the western and eastern continental sectors. Depth and temperature were the most influential variables in the different CCA analyses using datasets of molluscs with different lifestyles and from distinct classes, and sedimentological variables displayed a much more significant relationship with benthic molluscs than with demersal ones.

Keywords: Mollusc, assemblage, demersal, biodiversity, fisheries, Alboran Sea.

Introduction

The Mediterranean Sea is widely considered a marine biodiversity hotspot with more than 8500 species of marine macrooganisms in less than 0.8% of the world's oceanic area (Bianchi & Morri, 2000; Bouderesque, 2004; Coll *et al*., 2010). The Alboran Sea is located in the westernmost part of the Mediterranean Basin and considered a self-standing ecoregion by Spalding *et al*. (2007). Due to its oceanographic and ecological importance, with the confluence of Atlantic and Mediterranean water masses, this sea has frequently been highlighted as supporting one the greatest marine biodiversities along the European and North-African margins (Coll *et al*., 2010, García Raso *et al*., 2010; Templado, 2011). This great biodiversity includes a large number of commercial species that have led to the development of a wide variety of fishing techniques, as well as considerable fishing activity (García *et al*., 2012). In this context, bottom trawling is one of the most widely used fishing techniques and is responsible for the largest catches of demersal species in the northern Alboran Sea (Camiñas *et al*., 2004). This technique targets various fish species (e.g., *Merluccius merlucccius* and *Mullus barbatus*) and cephalopods (e.g., *Octopus vulgaris*) (Anon, 2010, García *et al*., 2012). Nevertheless, catches comprise a great diversity of faunal groups that are caught as by-catch during trawling operations. Among them, molluscs stand out for their high abundance and biomass in the hauls, as well as their exceptional diversity, with more than 1000 species of molluscs cited for the Alboran Sea (Gofas *et al*., 2011).

Modern fisheries management is trying to adopt an ecosystem-based approach that focuses more on ecosystem services rather than a single species (Pikitch *et al*., 2004; Bellido *et al*., 2011). Nevertheless, policies including an ecosystem approach to fisheries still present imprecise interpretations with regard to fisheries management and biodiversity conservation, as they are still in the very early stages of implementation in many countries (Jennings *et al*., 2014). The appropriate development of an ecosystem-based approach requires in-depth knowledge of the composition, structure, and distribution of biological communities from different fishing grounds, among other aspects. This type of information could help estimate, for instance, any potential changes that fishing activity could cause to the habitat and community structure (Thrush & Dayton, 2002; García *et al*., 2012). This is especially important in those basins with a high diversity of species and habitats in addition to significant fishing pressure, such as occurs in the northern Alboran Sea (Robles, 2010). Unfortunately, information on the circalittoral and bathyal communities of the Alboran Sea is still scarce, and this data is essential for implementing adequate conservation and management measures within the framework of current European Directives (e.g., the Marine Strategy Framework Directive) (Borja *et al*., 2010; Jennings *et al*., 2014).

Molluscs are one of the predominant groups in marine communities, contributing to the local biodiversity and representing an important food source for higher trophic levels (Hemminga & Duarte, 2000; Ballesteros, 2006; García Raso *et al*., 2010). Moreover, molluscs have been suggested, among other faunal groups, as proxies for assessing impacts or anthropogenic stress on total macrofaunal assemblages (Olsgard & Somerfield, 2000; Mendes *et al.,* 2007). Studies of molluscan assemblages in the northern Alboran Sea have mainly been carried out in the infralittoral zone (soft bottoms: Urra *et al*., 2011; seagrass meadows: Rueda *et al*., 2008, 2009; Marina *et al*., 2012; Urra *et al*., 2013; rocky bottoms: Urra *et al*., 2012), with limited information on certain groups/species (Salas, 1996; González & Sánchez, 2002) and assemblages of the circalittoral and bathyal zone (Gofas *et al*., 2014a; Rueda *et al*., 2015). Therefore, the aims of the present study are to: (1) characterise the composition and structure of molluscan assemblages of circalittoral and bathyal sedimentary bottoms in the northern Alboran Sea using otter-trawl fishing gear, based on species analyses according to their lifestyle (i.e., demersal or benthic species) and different classes (i.e., bivalves, gastropods, and cephalopods); (2) analyse the spatial distribution patterns of these assemblages; and (3) discover which environmental variables relate to the structure and distribution of the molluscan assemblages.

Material and Methods

Study area

The study area covers the northern Alboran Sea, from Punta Europa (Strait of Gibraltar) to Cabo de Gata (Almería), 350 km to the east, and includes Alboran Island (Fig. 1). The Alboran Sea is a biogeographical boundary for some organisms but it is also a transition zone between water masses from the Atlantic Ocean and the Mediterranean Sea. The eastern end of the Alboran basin is delineated by the imaginary line that runs between Cabo de Gata and Cape Fegalo (Oran, Algeria), separating typical Mediterranean waters from the Alboran Sea waters. The seafloor of the Alboran Sea is geomorphologically very complex and includes contouritic bottoms, seamounts, and submarine canyons with maximum depths down to 2000 m (Ercilla *et al.,* 1992). The meridional sector of the Alboran Sea is characterised by the presence of an old volcanic ridge oriented SW-NE, on which the Alboran Island is located (i.e., dorsal Alboran) (Vázquez, 2005).

Sandy and muddy bottoms alternate along the continental shelf of the northern Alboran Sea, which ranges in width from 4 to 20 km (Vázquez, 2005). On the shelf are areas of extensive sedimentary deposits from river run off

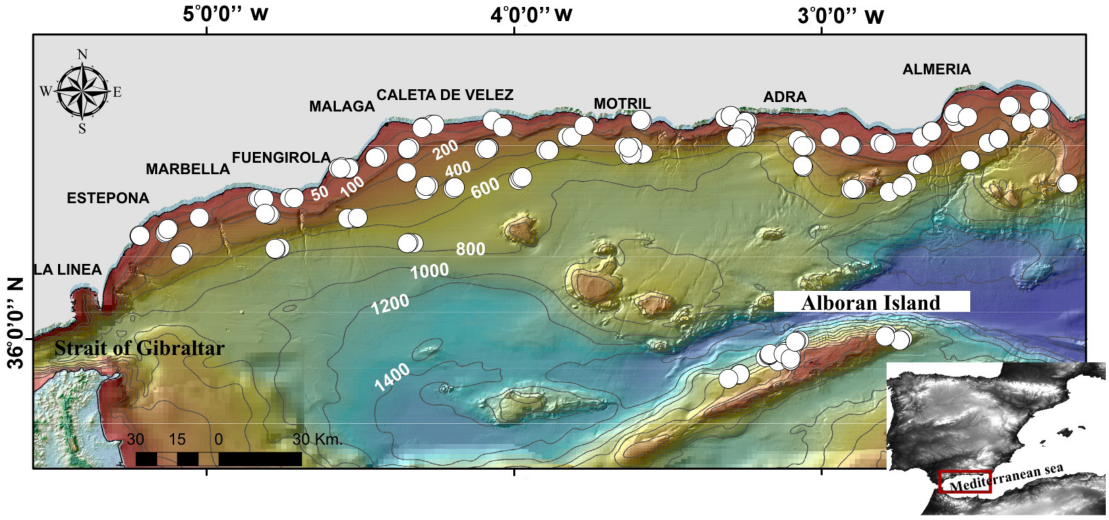

Fig. 1: Map of the study area in the northern Alboran Sea (southern Spain) showing the sampling stations (white points) where bottom otter trawl hauls were carried out during 2012 to 2015 MEDITS surveys.

(e.g., the Guadalmedina and Guadalhorce rivers in Málaga Bay) (Vázquez, 2005). On the continental slope, the soft bottoms are mainly composed of very fine sediments (Rey & Medialdea, 1989) whereas the shelf of Alboran Island is characterised by predominantly carbonate sedimentation of coarse materials, such as bioclastic remains (Hernández-Molina, 1993; Argudo, 2012). The complex dynamics of water exchange between the less-saline surficial Atlantic waters coming through the Strait of Gibraltar and the denser, saltier deep Mediterranean waters determine the particular hydrodynamic processes occurring in the Alboran Sea. These phenomena include the presence of upwelling waters rich in nutrients that generate abundant and high plankton productivity in the northwestern Alboran Sea. This productivity is higher than that of other Mediterranean areas and supports the development of many organisms, including commercially important species (Rodríguez, 1982; Sarhan *et al*., 2000; Vargas *et al*., 2007).

Sampling

A total of 190 hauls were performed on circalittoral and bathyal soft bottoms (30-800 m depth) along the northern Alboran Sea and around Alboran Island over four MEDITS surveys (International Bottom Trawl Surveys in the Mediterranean) carried out in spring from 2012 to 2015 (Fig. 1). Haul duration was determined as a function of depth, with 30-minute hauls for those stations located <200 m deep (continental shelf) and 60-minute hauls for those located >200 m deep (continental slope) (Bertrand *et al*., 2002). The geographical position of each haul was recorded using a Global Positioning System. A GOC-73 bottom trawl (average horizontal opening: 21.5 m; average vertical opening: 2.5 m; cod end mesh size: 20 mm) was used for this study (Fiorentini *et al*., 1999). Every specimen caught was identified to the lowest possible taxonomic level, counted and weighed (i.e., wet weight) on board. Further identification of the specimens in the laboratory was facilitated by either preserving the material in 70% ethanol or in the freezer at -20°C. Scientific names assigned to all taxa followed the nomenclature of the World Register of Marine Species (WoRMS). Temperature and salinity were recorded close the bottom (1-2 m above the seafloor) with a CTD SBE-37 coupled to the otter-trawl gear. Additionally, in the 2014 MEDITS survey, sediment samples were taken using a sediment collector coupled to the gear in order to study certain sediment characteristics (e.g., grain size) of the sampling stations and their relationship with the fauna.

Data analysis

Abundance (expressed as number of individuals) and biomass (expressed as weight) per species in each haul were standardised to 1 km^2 ($ind.\cdot km^{-2}$ and $kg.\cdot km^{-2}$, respectively). The frequency index (%F: percentage of hauls in which the species was present in relation to all hauls from the MEDITS surveys), as well as the dominance index based on abundance (%DN: percentage of individuals of a certain species over the total number of molluscs caught in all hauls from the study), and on biomass (%DB: percentage of biomass of a certain species over the total weight of molluscs caught in all hauls from the study), were estimated for each mollusc species.

The abundance and biomass data of each species from each haul of the four MEDITS surveys were pooled in a single abundance matrix and one biomass matrix that were analysed independently in order to identify potential assemblages. Due to the unbalanced capacity of the bottom otter trawl for catching the different molluscan species, analyses were carried out separately by grouping species that: (1) could display a similar catchability with regard to this sampling method, according to their lifestyle, such as demersal species (catches containing only demersal cephalopods) or benthic species (containing bivalves, gastropods and specific cephalopods that spend most of their time on the substrate); and (2) could belong to different molluscan classes (bivalves, gastropods and cephalopods). Unlike cephalopods, gastropods and bivalves were not present in every haul and therefore hauls with no gastropods or bivalves were not considered for molluscan class analysis. Further analyses including all molluscan species were performed to contrast the results of species with different lifestyles and those from different classes.

Previor to the multivariate analyses, the data was log (x+1) transformed in order to reduce the differences in abundance (for abundance data) and/or biomass (for biomass data) of highly dominant species (Clarke & Warwick, 2001). Subsequently, a resemblance matrix of all available samples for each analysis was obtained using the Bray-Curtis similarity index, and similarities were expressed with non-parametric multivariate classification (cluster) and ordination (nMDS) techniques (Clarke, 1993; Clarke & Warwick, 1994). The SIMPER procedure was applied to discover the contribution of each species to the dissimilarity between different assemblages according to lifestyle and molluscan class, and finally, combining the data for all mollusc species (Clarke & Warwick, 1994). A distance-based permutational multivariate analysis of variance (PERMANOVA) (McArdle & Anderson, 2001), based on the Bray-Curtis similarity matrix and applied to quantitative data (abundance and biomass), was used to test for significant differences between the assemblages observed, with sector (fixed factor, four levels) and depth (fixed factor, three levels) as sources of variation. Pairwise comparisons between the different sectors and depth were also carried out. The multivariate analyses were executed using the PRIMER6 & PERMANOVA+ software.

For the main assemblages observed, abundance (N), biomass (B), species richness (S), Shannon-Wiener diversity index (H': log_2) (Krebs, 1989), evenness index (J´) (Pielou, 1969), and taxonomic diversity index (Δ^*) (Clarke & Warwick, 1998) were estimated using the

PRIMER v6.0 software. A non-parametric analysis of variance (Kruskal-Wallis test) was carried out to test differences in the estimated indices where the data did not fit the conditions for parametric analyses (e.g., Analysis of Variance). These analyses were carried out using the SPSS v15.0 software.

Four geographical sectors were considered in the spatial analysis: (1) a western sector, with hauls performed between Estepona and Málaga, characterised by a high level of Atlantic influence due to its proximity to the Strait of Gibraltar and the presence of almost permanent upwelling (Sarhan *et al*., 2000); (2) a central sector, between Málaga and Castell de Ferro, with less influence from the western anticyclonic gyre and surficial Atlantic waters, in addition to limited upwelling; (3) an eastern sector, from Castell de Ferro to Cabo de Gata, with a greater influence of typical Mediterranean waters; and (4) an insular sector, corresponding to the Alboran Island sector, far from the continental margin and to a certain extent influenced by the eastern anticyclonic gyre.

For abiotic variables, the average near-bottom temperature and salinity, obtained with a CTD coupled to the gear, was estimated for each haul, and a Kruskal-Wallis test was run to test differences between these values in the molluscan assemblages. Grain size analyses of sediments were performed on 100 g subsamples taken from each dry sediment sample after its homogenisation. The sediment was sieved over a column of sieves (>2, 1, 0.5, 0.25, 0.125, and 0.063 mm), weighing the fractions retained on each sieve and obtaining the weight percentage for each fraction. The nomenclature of the sediment texture was based on the triangular Folk diagram (1954), defined by the ratios of gravel, sand, and mud. The sediment variables were analysed statistically using GRADISTAT software (Blott & Pye, 2001).

The relationships between environmental variables and mollusc abundance data were only analysed for the 2014 MEDITS survey, using a Canonical Correspondence Analysis (CCA) (Ter Braak, 1986). The statistical significance of the effect of each variable was tested with a Monte Carlo permutation test. Prior to this, the environmental variables were screened and those which presented a correlation of more than 0.9 in Spearman correlation analyses were not further considered. Environmental data expressed as a % was log (x + 1) transformed. The CCA analysis was performed using the R software package, considering matrices containing separate data for demersal, benthic, bivalve, gastropod, and cephalopod species, as well as a matrix with total mollusc data.

Results

Composition and structure of the mollusc fauna

The analysis of the bottom otter trawl hauls showed an overall dominance of fish when considering number of species (31% of all species), number of individuals (68% of all individuals), and biomass (86% of the total biomass). Molluscs were represented by 101 species (20% of all species), and constituted the second most abundant faunal group in terms of biomass (8%), and the third for abundance (6%), just after crustaceans (16%) (Annex 1 Supplementary Material). Other less represented faunal groups included echinoderms, annelids, and cnidarians.

The abundance and biomass of molluscs presented mean values of 2939.5 ± 1018 ind.·km^{-2} and 1606.1 ± 204 kg.·km^{-2}, respectively. Gastropods were the most well represented class with 41 species from 28 families, followed by cephalopods (34 spp. from 12 families), and bivalves (27 spp. from 15 families) (Annex 1 Supplementary Material). The greatest number of species were from the Sepiolidae family (9 spp.), followed by Loliginidae, Octopodidae, and Veneridae (5 spp. from each family). The squids *Abralia veranyi* (22% of all mollusc individuals) and *Alloteuthis media* (13%), as well as the bivalve *Neopycnodonte cochlear* (14%) were the most abundant individual species, whereas the cephalopod *O. vulgaris* (47% of the total mollusc biomass) and the bivalve *Venus nux* (9%) presented the highest biomass. The squids *Todarodes sagittatus* (51% of occurrence in all hauls), *Illex coindetii* (50%), and *A. veranyi* (44%) were the most frequently captured species over the four surveys, whereas 41 species (e.g., *Charonia lampas* and *Ocenebra erinaceus*) were caught in only one or two hauls. There were 44 species that occurred only on the shelf (e.g., *Arca tetragona* and *Turritella communis*), 25 species that were exclusive to the slope (e.g., *Euspira grossularia* and *Bathypolypus sponsalis*), with 14 of these found only below 350 m depth, and 33 species caught on both the shelf and slope (e.g., *Sepia orbignyana* and *Xenophora crispa*) (Annex 1 Supplementary Material).

Affinity between samples

Cluster and nMDS analyses using abundance data for demersal species (i.e., only cephalopods) and benthic species (mainly bivalves and gastropods together with specific cephalopods that generally occur on the seafloor) separately revealed two main groups of samples, with one of these divided into two subgroups. Group A corresponded to a shelf assemblage at 30-200 m depth; subgroup B1 corresponded to an upper slope assemblage at 201-350 m depth; and subgroup B2 corresponded to a middle slope assemblage at 351-800 m depth (Fig. 2A-B). SIMPER analyses showed the greatest dissimilarities between the shelf and middle slope assemblages using the demersal species data (av. diss.>91%) and benthic species data (av. diss.> 97%). In the demersal species analyses, the cephalopods *A. media* and *I. coindetii* characterised the shelf assemblage (group A), whereas *T. sagittatus* and *A. veranyi* characterised the upper and middle slope assemblages (subgroups B1 and B2). For benthic species, the cephalopod *O. vulgaris* and the bivalve *N. cochlear* characterised the shelf assemblage with similar contributions, whereas *S. orbignyana* and the cephalopod *Eledone cirrhosa* characterised the upper slope assemblage and the gastropod

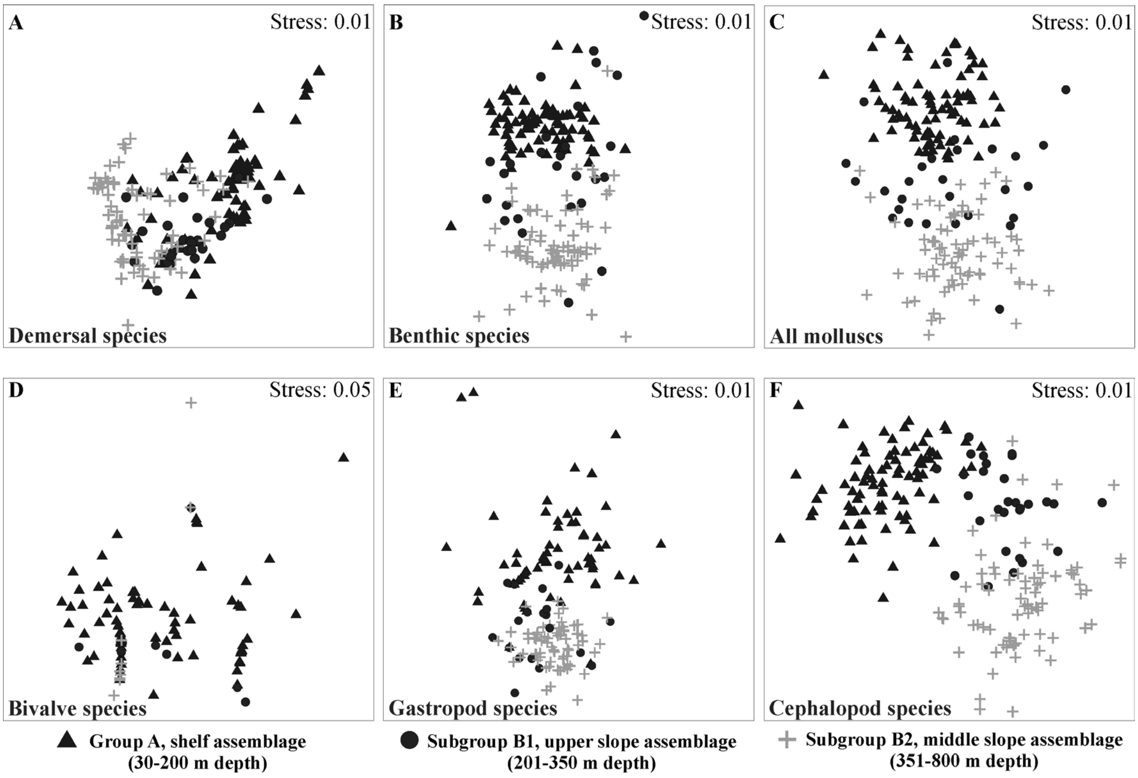

Fig. 2: Non-metric multidimensional scaling (nMDS) ordination of samples using the Bray-Curtis similarity index based on abundance data of molluscs species considering separate demersal (A), benthic (B), bivalve (D), gastropod (E) and cephalopod species (F) as well as total mollusc species (C) collected on circalittoral and bathyal soft bottoms of the northern Alboran Sea during 2012 to 2015 MEDITS surveys.

Galeodea rugosa and the cephalopod *B. sponsalis* characterised the middle slope assemblage, again with similar contributions.

Multivariate analyses of the different molluscan classes also displayed similar depth-related trends with two main groups (Group A, 30-200 m depth, shelf assemblage; Group B, 201-800 m depth, slope assemblage), but a further subdivision of group B into two subgroups (201-350 m depth, upper slope assemblage; and 351-800 m depth, middle slope assemblage) was detected for cephalopods (Fig. 2D-F). The strongest bathymetric differences were detected between the shelf and slope assemblages in SIMPER analyses (>66% in all molluscan classes), particularly between the shelf and the middle slope assemblages for gastropods and cephalopods (gastropods: av. diss. > 97.49 %; cephalopods: av. diss. > 95.21%; bivalves: av. diss. > 75.93%). The depth-related differences were mainly related to the higher abundance of (1) *N. cochlear* and *V. nux* on the shelf than in slope assemblages in analyses with bivalve data; (2) *X. crispa* on the shelf and *G. rugosa* in slope assemblages when considering gastropods and (3) *A. media, O. vulgaris* and *I. coindetii* on the shelf, *A. veranyi* on the upper slope and *T. sagittatus* and *B. sponsalis* in middle slope assemblages when considering cephalopods.

Finally, multivariate analyses combining the data for total mollusc species resulted in similar groups of samples as in the previous analyses. In this case, the SIMPER analyses displayed the highest differences between the shelf and the middle slope assemblages (av. diss.>97%). Overall, these differences were mainly due to the higher abundance of: (1) *O. vulgaris* and *N. cochlear* in the shelf assemblage; (2) *S. orbignyana* in the upper slope assemblage; and (3) *G. rugosa* and *B. sponsalis* in the middle slope assemblage, among other species.

SIMPER analyses using data from demersal and benthic species, different molluscan classes and total molluscs displayed a high dissimilarity in the shelf (30-200 m depth) and upper slope assemblages (201-350 m depth) in relation to geographical sectors, with maximum differences between the insular sector and the continental sectors (av. diss. generally between 70-85%). In the shelf assemblage, this dissimilarity was mainly due to a higher abundance of: (1) the cephalopods *S. orbignyana, A veranyi, Loligo vulgaris,* and *Loligo forbesii,* the bivalves *N. cochlear* and *A. tetragona,* and the gastropod *X. crispa* in the insular sector; and (2) the cephalopods *A. media, Sepia elegans, Sepia officinalis, S. oweniana,* and *I. coindetii*, the gastropod *T. communis*, and the bivalve *V. nux* in the continental sectors. In the upper slope assemblage, this dissimilarity was mainly due to a higher abundance of: (1) the cephalopods *S. orbignyana, L. forbesii, Rossia* *macrosoma* and *B. sponsalis* in the insular sector; and (2) the cephalopods *I. coindetii, E. cirrhosa*, *S. oweniana, E. moschata, R. minor, A. veranyi,* and *T. sagittatus*, along with the bivalve *V. nux* in the continental sectors.

The PERMANOVA test revealed stronger significant differences relating to depth than for the geographical sectors of the Alboran Sea when the analyses involved the separate data sets for each of the molluscan classes (Table 1). Nevertheless, the analysis using bivalve data resulted in the lowest Pseudo-F values (Table 1, Annex 2 Supplementary Material). Pairwise comparisons after PERMANOVA revealed that: (1) differences between sectors were consistently significant in the shelf and the upper slope assemblages ($p< 0.05$ in all cases); (2) the largest differences were sometimes detected between the insular sector (Alboran Island) and the western and eastern continental sectors; and (3) middle slope differences were only significant between benthic components of the insular sector and those of the western and/or central continental sectors ($p< 0.05$) (Table 1, Annex 2 Supplementary Material).

Table 1. Results of the PERMANOVA analyses testing differences between four sectors of the northern Alboran Sea (western, central, eastern and insular sector) and depth for separate demersal, benthic, bivalve, gastropod and cephalopod species as well as for all molluscan species.

	Source	**df**	**MS**	**Pseudo-F**	***p***
Demersal species	Sector	3	7285.2	4.5	0.001
	Depth	2	60294	37.57	0.001
	Sector × Depth	6	3913.3	2.4	0.001
	Residual	174	1604.8		
	Total	185			
Benthic species	Sector	3	10864	4.7	0.001
	Depth	2	74982	33.0	0.001
	Sector × Depth	6	5916.6	2.6	0.001
	Residual	178	2266.1		
	Total	189			
Bivalves	Sector	3	5849	2.4	0.012
	Depth	2	7252.8	3.0	0.006
	Sector × Depth	6	4387.3	1.8	0.018
	Residual	88	2409.8		
	Total	99			
Gastropods	Sector	3	9197.7	4.7	0.001
	Depth	2	44663	33.0	0.001
	Sector × Depth	6	5987.2	2.6	0.001
	Residual	150	19.932		
	Total	161			
Cephalopods	Sector	3	7167.9	4.5	0.001
	Depth	2	78339	49.9	0.001
	Sector × Depth	6	3418	2.1	0.001
	Residual	178	1566.9		
	Total	189			
All molluscs	Sector	3	8281.8	4.3	0.001
	Depth	2	72236	37.6	0.001
	Sector × Depth	6	4583.4	2.4	0.001
	Residual	178	1922.2		
	Total	189			

Ecological indexes

Mean abundance (N) of individuals, biomass (B), and species richness (S) values displayed significant decreases with depth (Kruskal-Wallis [K-W]: X^2= 100.3 for N, X^2= 103.7 for B, X^2= 35.4 for S; $p< 0.01$ in all cases), with a higher contribution of cephalopods in all assemblages, especially on the slope (> 200 m depth) (Fig. 3A-C). The Shannon-Wiener diversity index displayed similar mean values for the different assemblages (K-W: X^2= 4.1; $p> 0.05$), whereas the evenness index significantly increased in the slope assemblages (> 200 m depth) (K-W: X^2= 39.4; $p< 0.01$) (Fig. 3D-E). Finally, the taxonomic diversity index displayed a significant minimum value in the upper slope assemblage (201-350 m depth) (K-W: X^2= 46.9; $p< 0.01$) (Fig. 3F).

Environmental variables and their relationships with molluscan assemblages

A large variability in the sediment texture, near-bottom temperature (generally 1-2 m above the seafloor), and salinity was observed in the three bathymetric levels corresponding with the three main assemblages previously detected (Table 2). Overall, a higher temperature, lower salinity, and wider variety of sediment textures (with a predominance of sandy mud bottoms) was seen at the shelf bottom. Salinity increased with depth, but the temperature and types of soft bottoms decreased, with predominantly muddy bottoms on the slope (Table 2). The forward selection of the Canonical Correspondence Analysis (CCA) using separate data for demersal and benthic species, selected depth, temperature and percentages of

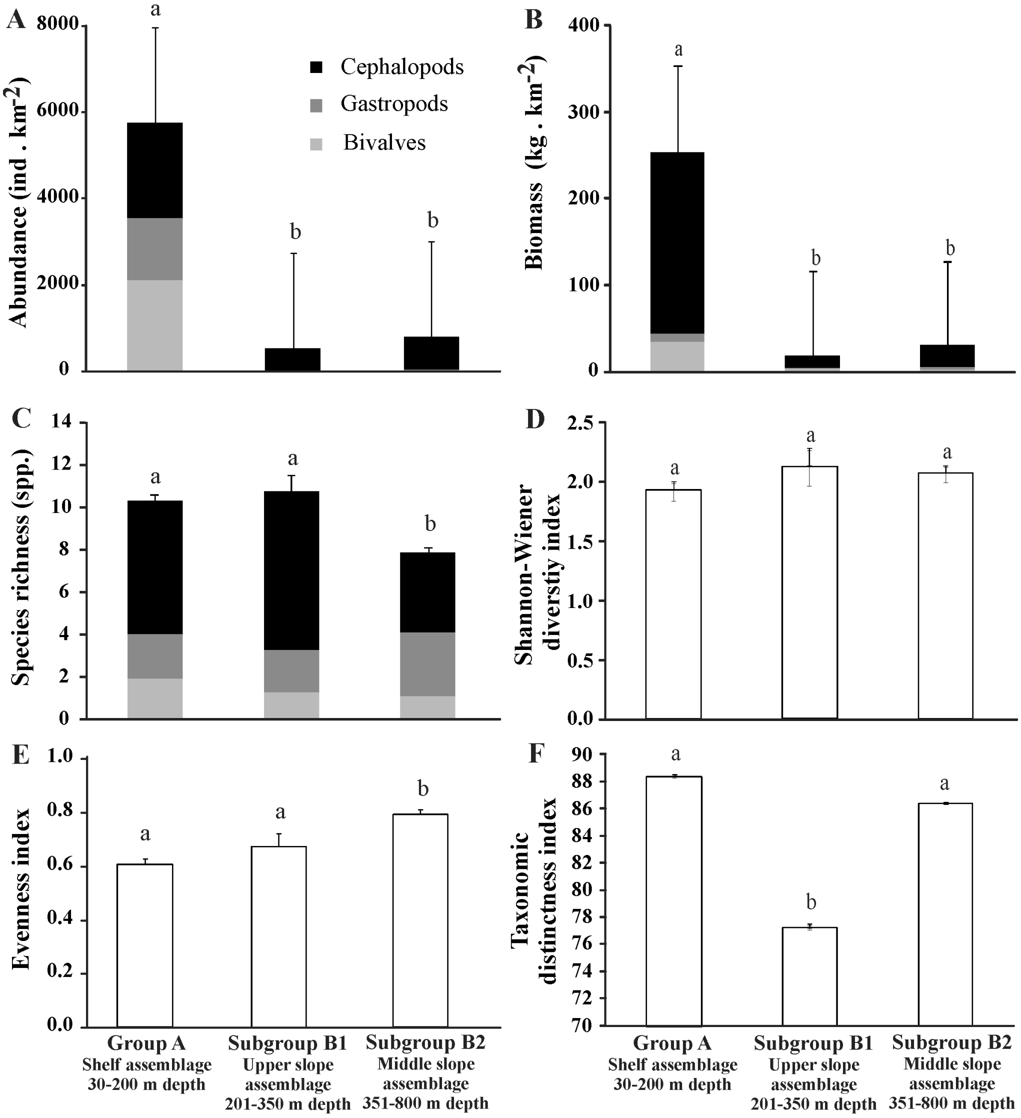

Fig. 3: (A) Abundance (ind.·km^{-2}), (B) Biomass (kg. km^{-2}) and (C) Species richness (S) (spp.) of molluscs, displaying the contribution of each molluscan class, (D) Shannon-Wiener diversity index, (E) Evenness index and (F) Taxonomic distinctness index in each of the main assemblages detected in the present study. Mean + SE. Letters above error bars display the results of Kruskal-Wallis pair-wise tests: different letters distinguish significantly different means at $p<0.01$.

Table 2. Depth, sedimentary and oceanographic characteristics of sampling stations monitored during the MEDITS_ES14 survey according to the main assemblages detected in the multivariate analyses. Results of the Kruskal-Wallis test between depth ranges are also displayed (**p< 0.01). Values are displayed as mean ± SE and range among brackets, except for sediment texture in which the percentage of occurrence of that sediment texture type within each depth range is displayed among brackets.

	Group A Shelf assemblage (30-200 m depth)	**Subgroup B1 Upper slope assemblage (201-350 m depth)**	**Subgroup B2 Middle slope assemblage (351-800 m depth)**
Depth (m)	89.20 ± 9.14	314.95 ± 14.53	598.90 ± 31.39
	(44.76 - 161.86)	(243.98 - 348.45)	(363.80 - 848.9)
Sediment texture	Sandy mud (42%)	Sandy mud (50%)	Mud (70%)
(% occurrence)	Muddy sand (26%)	Mud (25%)	Muddy sand (15%)
	Gravelly muddy sand (16%)	Sand (13%)	Sandy mud (15%)
	Gravelly sand (11%)	Muddy sand (12%)	
	Muddy sandy gravel (5%)		
Temperature (°C)**	13.98 ± 0.09	13.26 ± 0.02	13.16 ± 0.01
	(13.48 - 14.85)	(13.20 - 13.36)	(13.07 - 13.23)
Salinity (ppt)**	38.08 ± 0.12	38.41 ± 0.018	38.49 ± 0.002
	(37.70 - 38.23)	(38.32 - 38.46)	(38.46 - 38.51)

mud, very fine sand, and gravels for the benthic species analysis, but only depth, temperature and percentage of fine sand for the demersal species analysis (Fig. 4A-B, Table 3). The CCA analysis for demersal species explained a higher variance (39%) than that for benthic species (33%), with 26.2% of the assemblage variance and 79.6 % of the assemblages-environment variance for demersal species, and 22.9% of the assemblage variance and 39.9 % of the assemblages-environment variance for benthic species. On the other hand, CCA analyses using separate data for each molluscan class explained similar percentages of the variance (34%) for gastropods and cephalopods, but a lower percentage of the variance (29%) for bivalves. Considering the different CCA analyses, for cephalopods the closest correlations were found with depth and temperature, and for gastropods with depth and percentage of gravels. Finally, CCA analyses combining all molluscan species selected temperature, depth and different sediment features as the most important variables explaining the variance in the faunal data (Fig. 4C, Table 3). Axis I was mainly related to depth (negative correlation) and temperature (positive correlation), whereas axis II preferentially correlated with sediment features such as percentage of mud (positive correlation) and percentage of medium sand (negative correlation). The two axes were the most important in the CCA ordination, accumulating 37% of the variance, with 19.4% of the assemblage variance and 56.5% of the assemblage-environment variance (Table 3). The Monte Carlo test was significant for all the canonical axes of all the CCA analyses that were run (*p*< 0.001).

Discussion

This study has revealed a high species richness of molluscs that can be caught with an otter-trawl across circalittoral and bathyal sedimentary habitats in the northern Alboran Sea, when compared with similar studies using similar fishing gear (González & Sánchez, 2002; Quetglas *et al*., 2000; Serrano *et al*., 2006; Abad *et al*., 2007; Serrano *et al*., 2011; Quetglas *et al*., 2014). Moreover, the present study has increased the number of mollusc species known to be collected with this type of fishing gear in Spanish waters and the western Mediterranean Sea. Indeed, molluscs are one of the most diverse faunal groups collected with bottom otter trawl fishing gear in the area (101 taxa), and in the Mediterranean Sea as a whole. Cephalopods stood out due to their high species richness, abundance, and biomass in the hauls, with 40% of the 66 cephalopod species that occur in the Mediterranean Sea being caught (Bello, 2008; Sabelli & Taviani, 2014). This high representation in comparison with gastropods and bivalves is probably related to the demersal lifestyle of most cephalopods and to the sampling technique used in this study. This sampling technique generally captures species that live and feed near the seabed rather than species that occur only on the sediment (i.e., epibenthic species such as most gastropods and chitons) or within the sediment (i.e., endobenthic species such as most bivalves and scaphopods). Indeed, multivariate analyses (nMDS, PERMANOVA, CCA) performed for the present study using separate cephalopod data and demersal species data displayed more acute depth- and geography-related

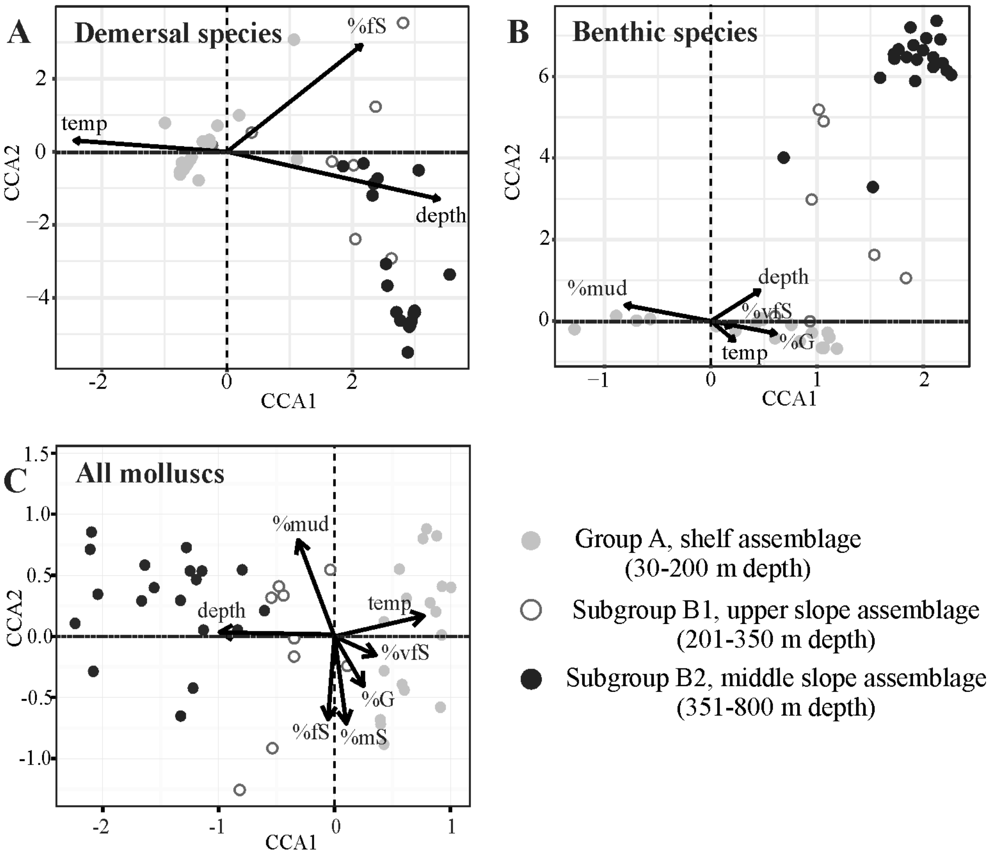

Fig. 4: Results of the canonical correspondence analysis (CCA) between the environmental variables (arrows) and the molluscan abundance data (circles) for demersal molluscs (A), benthic molluscs (B) and total molluscs combined (C). %mud: percentage of mud; temp: temperature; %vfS: percentage of very fine sand; %G: percentage of gravel; %mS: percentage of medium sand; %fS: percentage of fine sand.

Table 3. Results of the canonical correspondence analysis (CCA) for molluscs considering separate analyses for the benthic and for the demersal species as well as for all molluscs caught with otter trawl in circalittoral and bathyal soft bottoms of the northern Alboran Sea. Only those environmental variables showing significant effects (*p<0.05*) on molluscan assemblages are presented.

	Demersal species		**Benthic species**		**All molluscs**	
Environmental variables	**Axis I**	**Axis II**	**Axis I**	**Axis II**	**Axis I**	**Axis II**
Depth	-0.98	0.03	0.51	0.83	0.92	-0.35
Temperature	0.77	0.16	0.24	-0.53	-0.66	0.08
% gravel	0.24	-0.40	0.68	-0.33		
% medium sand	0.10	-0.71				
% fine sand	-0.05	-0.67			0.58	0.80
% very fine sand	0.35	-0.15	0.21	-0.14		
% mud	-0.3	0.78	0.89	0.43		
Eigen values	0.74	0.35	0.76	0.32	0.76	0.32
Species-environment correlations	0.96	0.89	0.97	0.95	0.93	0.82
Cumulative percentage variance						
of species data	13.1	19.4	12.1	22.9	18.4	26.2
of species-environment relation	38.3	56.5	39.9	75.3	56.0	79.6

trends than for other groups. This is likely to be due to the high contribution of these groups to the samples because of their greater catchability when compared to benthic species such as bivalves and gastropods.

The distribution of the main molluscan assemblages identified on the shelf and upper slope in the northern Alboran Sea is primarily linked to depth, and this study may be interpreted as a preliminary contribution in the detection of further assemblages on the shelf and slope related to sediment types and other environmental and anthropogenic variables. Nevertheless, the general spatial distribution pattern observed in the different analyses for the different groups of species resulted in two main assemblages being identified at depths of 30-200 m (shelf) and 201-800 m (slope), with a further subdivision (201-350 m depth, upper slope assemblages; and 351-800 m depth, middle slope assemblages) of the deeper assemblage identified in some analyses, which is similar to those previously reported for different faunal groups in the western Mediterranean Sea (fish: García-Ruiz *et al*., 2015; Ramírez-Amaro *et al*., 2015; decapods: Abelló *et al*., 1988; cephalopods: Quetglas *et al*., 2000; González & Sánchez, 2002; megabenthic fauna: Abad *et al*., 2007). Moreover, the existence of an upper slope assemblage in some of the analyses can be interpreted as a transition zone between shelf and slope assemblages, as detected at a similar depth range in other studies on cephalopods and fish, with the coexistence of species that display a wide bathymetrical distribution from adjacent bathymetrical areas (Quetglas *et al*., 2000; González & Sánchez, 2002; García-Ruiz, 2012).

The segregation of assemblages between the shelf and the slope is probably due to the response of organisms to changes in various environmental variables that are partly influenced by depth, including features of the water column (e.g., seawater temperature, food availability, and pressure) and sediment (e.g., grain size and organic matter content), in addition to ecological interspecific interactions which may also influence the bathymetric ranges of some species (Snelgrove & Butman, 1994; Cartes *et al*., 2004; Martins *et al*., 2014). In this context, some of the Canonical Correspondence Analyses (CCA) showed that temperature and depth, as well as certain sediment-related variables, are important for explaining the distribution of mollusc assemblages, as observed in littoral studies of molluscs and decapods carried out in the northern Alboran Sea (García Muñoz *et al*., 2008; Urra *et al*., 2011). Temperature always correlated with axis I of the CCA, and sometimes less dispersion of slope samples was detected along this axis which could be related to the thermally stable environment below 200 m in the Mediterranean Sea (Hopkins, 1985). In the study area, there was an increased percentage of mud and a decrease in the types of sedimentary bottoms with depth, as reported for many deep areas worldwide (Tyler, 2003). This could explain the higher dispersion of shelf samples in some analyses as a result of a higher environmental heterogeneity of the shelf in comparison to the slope (Table 2). For demersal species such as cephalopods, sediment texture variables did not display a strong relationship with the distribution of assemblages in the CCA. This could be due to their relative independence from the substrate, unlike depth (González & Sánchez, 2002).

The different assemblages detected were characterised by certain mollusc species that occurred at specific depth ranges (Annex 1 Supplementary Material). In terms of abundance values the shelf assemblages (30-200 m depth) were dominated by the cephalopods *A. media* and *O. vulgaris* and the bivalves *N. cochlear* and *V. nux*, whereas the slope assemblages (201-800 m) were basically dominated by the cephalopods *T. sagittatus*, *A. veranyi*, *S. orbignyana,* and *E.cirrhosa* at depths of 200-350 m; and *T. sagittatus, B. sponsalis* and the gastropod *G. rugosa* dominated at depths of 350-800 m. Some of these cephalopod species are similar to those of the main cephalopod assemblages detected along the Mediterranean margin of the Iberian Peninsula using bottom otter trawl gear (González & Sánchez, 2002). Regarding biomass, *O. vulgaris* dominated the shelf and *T. sagittatus* dominated the upper slope, as also appears to occur in other western Mediterranean locations (e.g., the Balearic Sea) (Quetglas *et al*., 2000). The composition of the mollusc assemblages also displayed bathymetrical differences, with a larger component of exclusive species found on the shelf (44 spp.) compared to the middle slope (14 spp.). This could be promoted by the higher heterogeneity of bottom types on the shelf, as mentioned above, because several mollusc species are linked to specific sedimentary habitats (Gofas *et al*., 2011). With regard to conservation, some of the species collected with bottom otter trawl methods in the Alboran Sea are found on various protected species lists, including *C. lampas* (Berne Convention [Annex II], Barcelona Convention [Annex II], Habitat Directive [Annex V], Spanish and Andalusian List of Threatened Species [category: vulnerable]), and *Ranella olearium* (Berne Convention [Annex II], Barcelona Convention [Annex II], Andalusian Red List for Invertebrates [category: vulnerable]). The gastropod *C. lampas* was only collected in the Alboran Island sector, probably due to the lower fishing pressure in this area. The populations of this gastropod near Alboran Island could be crucial for its conservation in the Alboran Sea as it is in serious decline along the continental margin of the Iberian Peninsula (Templado, 1991).

The shelf assemblage (30-200 m depth) was characterised by high abundances and biomasses of molluscs that decreased markedly with depth. This contrasts with observations made for fish assemblages in the same depth ranges in the Alboran Sea where, despite decreased abundance, the fish biomass increases sharply from a depth of 500 m due to the larger size of the individuals sampled (García Ruiz *et al*., 2015). The lower biomass of molluscs observed with depth is related to the capture of less abundant species that are smaller than those on the shelf -this seems to be common for molluscs in general and especially cephalopods in the Western Mediterranean (Quetglas *et al.*, 2000; González & Sánchez, 2002; Gofas *et al.*, 2011). The lower mean values for the Shannon-Wiener diversity index reported here compared to those of similar studies carried out in the southern Iberian Peninsula are probably related to the fishing gear used for collecting the faunal samples. This study involved a bottom otter trawl and there was a notably lower number of gastropod and bivalve species, as well as a dominance of cephalopods in the samples analysed. In contrast, in studies using a benthic dredge, a higher number of species was reported, with much higher diversity index values (>2 bits) (Rueda & Salas, 2003; Urra *et al.*, 2011; Díaz *et al.*, 2017). Some of the species frequently reported in soft-bottom molluscan assemblages of the Western Mediterranean Sea were observed in low abundances and frequencies in this study due to: (1) their preferential bathymetric range, which is shallower than the depth range of this study (e.g., *O. erinaceus* usually inhabits littoral soft bottoms) (Urra *et al.*, 2011); (2) their small size that prevents their capture with otter trawls, as observed for *Tritia ovoidea*, *Nucula sulcata* and Philinidae species (Gofas *et al.*, 2011; Díaz *et al.*, 2017); (3) their association with other organisms that are not abundantly collected with a bottom otter trawl (e.g., *Pseudosimnia adriatica* associated with unknown alcyonaceans or *Armina tigrina* associated with pennatulaceans) (Oliverio & Villa, 1995; Gofas *et al.*, 2011); (4) their preference for habitats that are usually non-trawlable (e.g., *Anomia ephippium* lives fixed to rock or other hard substrates) (Gofas *et al.*, 2011); and (5) the pelagic lifestyle of certain species, such as *Heteroteuthis dispar*, which can migrate hundreds of metres vertically in search of food close to the surface (Guerra, 1992).

The molluscan assemblages displayed geographical differences in composition and structure between the insular sector (Alboran Island) and the continental margin. Nevertheless, these differences among sectors were less acute in the middle slope, probably due to higher environmental similarities between sectors in the deep areas. These differences may be related to the geographical location of Alboran Island and some of its related features (e.g., it is far from coastal activities and sewage or river discharge, with some habitats having a higher diversity and being healthier). The differences could also be related to low fishing pressure, which is limited to the deep sea bottom near Alboran Island. This could influence the segregation of the samples collected in shallower stations in this area from those collected along the continental margin. The low abundance of certain species observed near Alboran Island, which are typically predominant on the continental margin, could be related to their trophic importance as prey. This would include the smaller cephalopods *A. media* and *S. oweniana*, which are usually predated by the demersal fish community (Velasco *et al.*, 2001). The higher biodiversity and lower fishing pressure near Alboran Island would promote a higher number of fish predators and predation pressure in comparison to other areas of the northern Alboran Sea. Other species were more frequently collected near Alboran Island due to their habitat preference, for example *A. tetragona* and *N. cochlear*, which generally inhabit bioclastic bottoms. These types of bottoms are more common at 80-200 m depth on the Alboran Island shelf than along the continental margin of the Iberian Peninsula where large rivers induce higher seafloor sedimentation rates (Gofas *et al.*, 2014b). Moreover, the presence of dense aggregates of *N. cochlear* at depths of more than 80 m (Peñas *et al.*, 2006) would support high abundances of *Monia patelliformis* and *Chama circinata*, which can settle inside the shells of large bivalves, and which were also collected in high numbers at this depth range in the area.

Finally, this study suggests the importance of combining biodiversity study methods and further monitoring for the Marine Strategy Framework Directive, to provide complete information on the demersal and benthic assemblages, including those comprising molluscs. In this context, some mollusc species present in the sampled areas were collected with a beam trawl (Rueda *et al.*, 2015) but not with an otter trawl due to the different catchability of the distinct groups of molluscs. The latter may therefore provide valuable information on epibenthic molluscs and cephalopods, but for other classes (e.g., bivalves) it would be necessary to use sampling methods that target epifauna and infauna (Moya-Urbano *et al.*, 2015). This could explain why multivariate analyses (CCA, MDS, PERMANOVA) using only bivalve data did not present acute differences between assemblages compared to those using cephalopod or even gastropod species, because the bivalve catchability with an otter trawl is much lower than that of cephalopods and some gastropods. The molluscan assemblages detected in this study may represent a first step, with further studies using methods that target the epifauna and endofauna being needed in order to disentangle all the potential assemblages that may occur within the main assemblages detected in this study.

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