Drivers of variation in seagrass-associated amphipods across biogeographical areas

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ABSTRACT

Amphipods are one of the dominant epifaunal groups in seagrass meadows. However, our understanding of the biogeographical patterns in the distribution of these small crustaceans is limited. In this study, we investigated such patterns and the potential drivers in twelve *Cymodocea nodosa* meadows within four distinctive biogeographical areas across 2000 Km and 13° of latitude in two ocean basins (Mediterranean Sea and Atlantic Ocean). We found that species abundances in the assemblage of seagrass-associated amphipods differed among areas following a pattern largely explained by seagrass leaf area and epiphyte biomass, while the variation pattern in species presence/absence was determined by seagrass density and epiphyte biomass. Seagrass leaf area was also the most important determinant of greater amphipod total density and species richness, while amphipod density also increased with algal cover. Overall, our results evidenced that biogeographical patterns of variation in amphipod assemblages are mainly influenced by components of the habitat structure, which covary with environmental conditions, finding that structurally more complex meadows harboring higher abundance and richness of amphipods associated.

1. Introduction

A primary goal in ecological studies is to determine how biological diversity, in terms of the composition and abundance of species, is distributed, and which are the processes that drive these patterns (Gray, 1997; Witman et al., 2004; Máñez-Crespo et al., 2020). This goal is particularly relevant in the current context of global change, biodiversity loss and ecosystem fragmentation. The heterogeneity and complexity of marine habitats often play a key role in mediating biodiversity (Moore and Hovel, 2010; Staveley et al., 2017; Navarro-Mayoral et al., 2020). Such is the case of habitats underpinned by 'ecosystem engineering' species (*sensu* Wright and Jones, 2006), as reported elsewhere (e.g., Connell, 1978; Alsaffar et al., 2020). Vegetated habitats are known to support greater species diversity and abundances than adjacent unvegetated habitats (Vázquez-Luis et al., 2009; Fonseca et al., 2011; Schmidt et al., 2011; Urra et al., 2017), with both diversity

and abundance of biological assemblages usually increasing with habitat availability and complexity (Navarro-Mayoral et al., 2020). Structurally complex marine habitats, such as seaweed (i.e., kelp or fucoids) forests and seagrass meadows, however, are declining at local, regional and global scales (Thomson et al., 2015; Ferrari et al., 2016; Unsworth et al., 2018; Dunic et al., 2021; Turschwell et al., 2021; but see de Los Santos et al., 2019), which may have consequences on diversity and abundance patters of associated fauna.

Seagrasses are widespread 'ecosystem engineers' on coastal soft bottoms, where they provide key ecological functions and ecosystem services, such as contributing to coastal protection (Fourqurean et al., 2012; Ricart et al., 2015), act as carbon sinks (Duarte et al., 2005; Bañolas et al., 2020), or support a large biodiversity of associated fauna (Tuya et al., 2014; Thomson et al., 2015; Salo and Gustafsson, 2016). Regarding the later ecological function, the structural complexity of seagrass meadows has been directly linked with an increase in the

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density and diversity of associated epifauna (e.g., crustaceans and gastropods) and macroalgae (Moore and Hovel, 2010; Gartner et al., 2013). Whilst seagrass physical structure modifies local environmental conditions and biotic interactions, environmental factors, such as exposure to hydrodynamics or seawater temperature, may also influence the structure of the seagrass habitat (McDonald et al., 2016; Mtwana Nordlund et al., 2016). This may have cascading effects on the diversity and abundance of certain seagrass-associated epifaunal groups, such as crustaceans (Boyé et al., 2017), as well as on species interactions across biogeographical scenarios (Tuya et al., 2019; Martínez-Crego et al., 2021).

Seagrass canopies provide food and shelter against predators for a range of epifaunal organisms, such as amphipods, polychaetes, and gastropods (Bartholomew, 2002; Carr et al., 2011). Among seagrass-associated epifauna, amphipods are one of the most abundant (Hyndes and Lavery, 2005; Vázquez-Luis et al., 2009; Sweatman et al., 2017; Lin et al., 2018) and diverse taxocenosis (Gambi et al., 1992; Michel et al., 2015). Such small crustaceans show a great variety of feeding habits, entailing suspensivorous, detritivorous, predators, herbivorous, or omnivorous (Viejo, 1999; Goecker and Kåll, 2003; Guerra-García et al., 2014). Amphipods play a major role in the transfer of energy towards higher trophic levels (Myers and Heck, 2013; Sturaro et al., 2014), serving as a direct food source for carnivorous decapods and fish (Pinnegar and Polunin, 2000).

Cymodocea nodosa (Ascherson, 1869) is a dominant seagrass in subtidal zones across the Mediterranean Sea and the adjacent Atlantic Ocean, including southern Portugal, Mauritania, the Canary Islands and Madeira (Masucci et al., 2012; Tuya et al., 2021). *Cymodocea nodosa* meadows encompass different ecoregions, under varying environmental conditions and landscape configurations (Tuya et al., 2019; Máñez-Crespo et al., 2020). Thus, they provide an ideal case-study to investigate contrasting habitat structural complexities and environmental conditions as drivers of spatial variation in the distribution of seagrass-associated fauna across large (regional) scales.

This study aims to investigate patterns and drivers of variation in *C. nodosa*-associated amphipod assemblages across a wide biogeographical range, at the scale of 1000s of Km. Particularly, we examined which meadow attributes (i.e., seagrass leaf area and density, cover and richness of associated algae, and biomass of leaf epiphytes) and environmental conditions (i.e., sediment grain size, nitrogen availability, annual sea surface temperature, temperature and chlorophyll-a) contributed to explain the amphipod total density and species richness, as well as species presence/absence and abundances in amphipod assemblages. Although there is no consensus in the terms used to define habitat complexity or standardized methods to quantify it, measures traditionally used in aquatic vegetated system included macrophyte biomass and stem density (Kovalenko et al., 2012; Boström and Bonsdorff, 2000; McCloskey and Unsworth, 2015). Also, epiphytes growing on seagrass promote habitat availability and complexity by creating new space for settlement and shelter, as well as increasing food supply for the associated fauna (Hall and Bell, 1988; Murphy et al., 2021). However, the role in complexity provision of other structural elements that
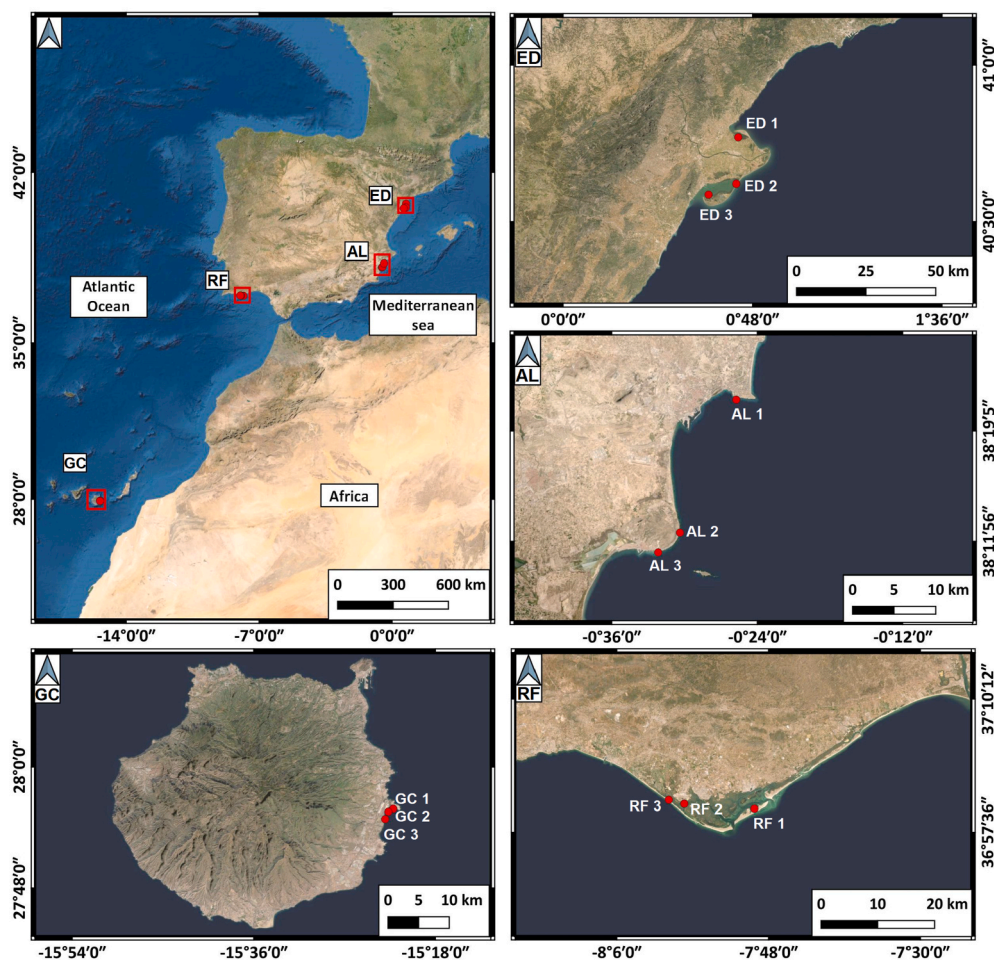

Fig. 1. Map of the four biogeographical areas, where amphipod assemblages and potential environmental and seagrass structure predictors were studied. The two areas in the NW Mediterranean are: Ebro Delta (ED) and Alicante (AL) and the two areas in the NE Atlantic are: Ria Formosa (RF) and Gran Canaria Island (GC). In each area, three meadows (red circles) were arbitrarily chosen to represent the variety of meadows within each area.

increase habitat availability such as plant shape or surface area is less clear (Kovalenko et al., 2012). Thus, in our study, we considered that seagrass leaf area increases habitat availability, while seagrass density, drifting macroalgae and seagrass epiphytes increase both, habitat availability and complexity.

2. Materials and methods

2.1. Study areas and sampling design

We conducted a simultaneous study in four biogeographical areas across a temperate to subtropical latitudinal gradient (ca. 2000 Km, 13° of latitude; Fig. 1), covering from the Northeaster (Ebro Delta [ED], 41° N) and Easter-Iberian Peninsula (Alicante [AL], 38° N) in the Western Mediterranean to the Southern-Iberian Peninsula (Ria Formosa [RF], 37° N) and Macaronesian (Gran Canaria Island [GC], 28° N) in the Northeastern Atlantic Ocean.

At each area, we sampled three shallow (<10 m depth) *C. nodosa* meadows, separated by 3–15 Km. Meadows were arbitrarily chosen to represent the variety of meadows within each biogeographical area. Sampling was conducted in summer (July 2014), when the plant growth is roughly stable after the late-spring peak in both the Mediterranean Sea and the Atlantic Ocean (Terrados and Ros, 1992; Tuya et al., 2006). We also performed sampling in all meadows within each area in the shortest possible interval (1–3 days; 10th-15th July), to focus on spatial variation avoiding the masking influence of seasonal variation in amphipod communities (e.g., Jacobucci et al., 2009).

2.2. Collection of amphipod assemblages

We collected 4 to 5 replicated samples of vegetation and associated epifauna, separated by ca. 5 m, by placing a fine mesh bag (<1 mm) affixed to a flexible circular hoop (25 cm diameter: ED; 26 cm diameter: RF), or quadrat (20 cm: GC and 22.5 cm: AL), over the seagrass canopy (0.040–0.053 m^2 of total area), which was then cut at the sediment surface level, following the methodology described by Martínez-Crego et al. (2021). Sample bags were transported to the laboratory, where amphipods were separated from other organisms retained by a mesh, identified, and counted under a stereomicroscope. For each sample, we determined species abundances (expressed as number of individuals per m^2) and species presence/absence of the amphipod assemblage. Total density and species richness, both expressed per m^2, were also calculated for each sample.

2.3. Habitat structure and environmental context

Attributes of meadow structure were measured in vegetation collected in the same mesh bags used for amphipod collections. In the laboratory, we quantified seagrass density per m^2 after counting the number of *C. nodosa* shoots. We also quantified the richness of the seagrass-associated macroalgae (i.e., number of species) and their algal cover (i.e., expressed as the area covered after spreading all algal species on a horizontal surface in cm^2 per m^2). The epiphyte biomass, expressed in mg of dry weight per g of seagrass leaf dry mass, was measured in 4–18 of those seagrass shoots collected in the mesh bags, after drying (60 °C for 48 h) epiphytes scraped off leaves. Seagrass leaf area per shoot was also measured in 20–22 undamaged shoots of *C. nodosa* randomly collected at each meadow, and expressed as leaf area excluding sheaths, in cm^2 per shoot.

To assess environmental conditions, we measured carbon (C) and nitrogen (N) content in plant tissues, as a proxy of environmental nitrogen availability, since it is a more integrative measure than discrete measurements of nutrient concentration in seawater samples (e.g., Martínez-Crego et al., 2006). C and N content were analysed in powdered dry samples of seagrass leaves without epiphytes (n = 5 replicates of pooled material from three shoots each), using a Carlo-Erba

elemental analyser (Instruments EA 1108), and expressed as % of dry weight on a molar basis. In each meadow, 5 sediment samples were collected using a 5 cm diameter core inserted 5 cm into the sediment. We measured sediment grain size and the proportion of fine and coarse fractions in each sample, after organic matter removal with diluted (6%) H$_2$O$_2$ and drying at 60 °C for 48 h. The grain size was expressed in phi units, defined as: φ = -log2 d (mm), where d is the particle diameter in mm. The sieving of the coarse (sand and gravel) and fine (clay and silt) fractions were carried out through a 0.062 mm mesh. The coarse fraction was mechanically sieved, at 1 φ intervals, from −4.00 φ (16 mm) to 4.00 φ (0.062 mm). Pipette analysis was performed at 1 φ intervals in the fine fraction, after the addition of 0.5% sodium hexametaphosphate solution as dispersant.

We retrieved, for each meadow, estimates of monthly sea surface temperature (Reynolds Optimum Interpolation Sea Surface Temperature provided by NOAA), chlorophyll-a (multiple-satellite cross-calibrated chlorophyll product provided by NASA), available using the NAUPLIUS Spatio-temporal Data Toolkit (https://www.st.nmfs.noaa.gov/copepod/about/about-nauplius.html). This toolkit tackled the different spatial grid sizes (from 9 km to 1°) and reference systems used by the original data sources. Estimates were retrieved using exact meadow coordinates (latitude and longitude). We used the retrieved time series to average, for the period 2000–2014, the annual sea surface mean temperature and range, as well as the annual mean chlorophyll-a. Such environmental variables were chosen as geographical predictors, since they mostly vary among biogeographical areas.

2.4. Statistical analyses

2.4.1. Patterns of variation in amphipod assemblages

Multivariate Generalized Linear Models (multivariate GLMs) were conducted to investigate differences between biogeographical areas, separately for the species abundances data and presence/absence in amphipod assemblages, using the 'mvabund' R package (Wang et al., 2012). Including the random factor 'meadow' was not possible, since this option is not currently available for multivariate GLMs. Such multivariate differences were visualized using non-metric multi-dimensional scaling bidimensional plots (nm-MDS), based on Bray Curtis dissimilarities and Jaccard distances for species abundances and presence/absence, respectively. For nm-MDS routines, we used the 'vegan' R package (Oksanen et al., 2019). In addition, indicator species analysis was performed using the 'indicspecies' R package (version 1.7.12), with the function 'multipatt', to identify indicator species for a given area (Cáceres and Legendre, 2009). In this approach, the indicator value combines the specificity and fidelity components of each species for each area, showing significant p-values, in our case, only when a species is completely restricted to a given area (specificity = 1; i.e., it appears in meadows of this area only) and it appears in all meadows belonging to this area (fidelity = 1). This is due to low replication (with only 3 meadows per area) and high between-meadow variability.

Mixed effects GLMs with 'area' as a fixed factor and 'meadow' as a random factor nested within 'area' (random intercept models) were used to examine univariate differences in amphipod density and species richness. For each response variable, we used a null-hypothesis significance approach, in which the full mixed model was compared, using a χ^2 likelihood ratio test, against the corresponding 'null' model with only the random effect corresponding to 'meadow' (Harrison et al., 2018). According to this approach, significantly different models denoted a significant effect of the fixed factor 'area'.

All GLMs were performed using the 'lme4' R package (Bates et al., 2015). In multivariate models, we used a 'negative binomial' error distribution for species abundances and a 'binomial' error distribution for species presence/absence, with a 'log' link function in both cases. In all mixed-effect models, we also used a 'negative binomial' error distribution with a 'log' link function, since we were dealing with over-dispersed count data (Buckley, 2014). Diagnosis plots of residuals and

Q–Q plots were used to visually inspect the appropriateness (i.e., linearity assumptions) of the fitted models (Harrison et al., 2018).

2.4.2. Predictor variables influencing amphipod assemblages

A set of GLMs were implemented to explore the relative contribution of the measured predictor variables on variation in species abundances and presences/absences (multivariate GLMs) and in amphipod total density and species richness (univariate GLMs). In order to focus on comparisons among biogeographical areas (i.e., dismissing small scale variation), we used the mean value of each meadow as replicates. Prior to implementation of the models, correlations (Spearman coefficients) among each pair of potential predictor variables were tested and visualized using the 'corrplot' R package (Wei et al., 2017). To limit the inclusion of over-correlated predictors in the models, we used two complementary criteria. Firstly, we chose that one with a larger biological significance among those predictors that were significantly correlated (Spearman correlation coefficient with p-value <0.05; see Supplementary Fig. S1, Table S1), following Bolker (2008). Then, we included seagrass leaf area (uncorrelated to other predictors), seagrass density (significantly correlated with seagrass C:N ratio and N content, latitude and sea temperature range), algal cover (correlated with algal richness), epiphyte biomass (significantly correlated with sea surface mean temperature, sediment grain size and fine sediment) and chlorophyll-a (correlated with seagrass C:N and N, latitude, sea surface mean temperature, and sea temperature range) as predictor variables. Secondly, variance inflation factors (VIF) of each predictor, calculated using the 'car' R package (Fox et al., 2012), indicate a high collinearity with other predictors included in the model when are higher than 10 according to Quinn and Keough (2002). In our case, included predictors that showed a VIF <10 in all models and none was excluded from the models according to the VIF criteria. Diagnosis plots of residuals and Q-Q plots were again used to visually inspect the appropriateness of the fitted models, while assumptions of homogeneous variances were checked as well using the Breusch-Pagan heteroscedasticity test.

For model selection in univariate responses (i.e., amphipod total density and richness), we firstly used the 'MASS' R package (Venables and Ripley, 2002) to perform a backward stepwise approach, by iteratively removing from the full model the predictor variable with the lowest contribution, until obtaining the most parsimonious model according to the Akaike Information Criterion (AIC). Lastly, we used the 'MuMIn' R package (Barton and Barton, 2015), with a double aim: (i) to validate the previous model (stepwise) selection by rank candidate models (i.e. models containing all combinations of 1, 2, 3 or 4 predictors), by the AIC corrected for small samples (AICc); and (ii) to estimate the relative importance of each predictor variable, including models ranked by the AICc, Adjusted R^2, p-values, and significant predictors for each model. In the case of multivariate amphipod responses (i.e., species abundances and presences/absences), for which 'MASS' and 'MuMIn' R packages are not available, we perform the model selection using a step-by-step procedure. We generate different models containing the predictor variable with the highest contribution in the full model, alone and in all possible combinations with the other predictors by pairs. Then, the most parsimonious model (with lowest AIC) was selected based on information criteria.

2.4.3. Patterns of variation in selected predictor variables

GLMs were also conducted to examine differences between biogeographical areas in predictor variables selected. Selected predictor variables encompassed those directly selected in the previous set of GLMs (i. e., seagrass leaf area, seagrass density, algal cover and epiphyte biomass), as well as for those variables that significantly correlated to them (i.e., algal richness, seagrass nitrogen, seagrass carbon: nitrogen ratio, latitude, sea surface temperature range and mean, sediment grain size and fine sediment; see Supplementary Table S1 and Fig. S1). These correlated variables are not included in the models built for amphipods to avoid collinearity, but differences between areas were explored to

interpret their influence.

3. Results

3.1. Patterns of spatial variation in amphipod assemblages

A total of 38 taxa were identified (28 determined at the species level, 8 at the genus level and 2 at the family level). Among them, 18 taxa were classified as detritivorous, 2 herbivorous, 13 detritivorous/herbivorous, 3 detritivorous/carnivorous, and 2 detritivorous/herbivorous/carnivorous according to our literature review (Supplementary Table S2). Thus, the amphipod community was dominated by detritivorous/herbivorous amphipods (i.e., 82%: 31 out of 38 total taxa). Multivariate GLMs showed that amphipod assemblages significantly differed among biogeographical areas in terms of species abundances and species presence/absence (Fig. 2, Table 1). Area to area comparisons in GLMs detected significant differences in species abundances only between ED and AL, while species presence/absence significantly differed between GC and the other areas (Supplementary Table S3).

Results from the indicator species analysis showed no representative species for ED (indicator value < 1), both in terms of species abundances and presences/absences (Supplementary Table S4). This is because the three taxa found only in ED (i.e., Ischyroceridae n.i, *Gammarus aequicauda* and *Corophium* sp.) were not present in all meadows in this area and showed high variation between meadows in their abundances (Supplementary Table S4). In contrast, for the rest of the areas (i.e., AL, RF and GC) different indicator species were identified, as strongly associated to each of them (indicator value = 1). We found that *Centraloecetes dellavallei* was indicator species for AL in terms of both, species abundances and presence/absence, exclusively and consistently appearing in all three meadows within this area (Supplementary Table S4). Also, *Ampelisca diadema, Microdeutopus stationis, Ampelisca brevicornis* and *Lysianassa costae* appeared exclusively in AL, but they were only present in one or two meadows within this area (not significant due to fidelity <1). Regarding species presence/absence in the assemblage, *Photis longicaudata* was the only significant indicator species for GC, while other species (not significantly representative) were specific to GC but not present in all meadows of this area (i.e., *Sunamphitoe pelagica, Pariambus typicus, Caprella caveidinae, Ampithoe rubricata, Maera grossimana* and *Marea* sp.) or showed fidelity to GC but were not exclusive to this area (i.e., *Phtisica marina* and *Pseudoprotella phasma)*. In RF three indicator species were found, Aoridae n.i, *Iphimedia* sp. and *Caprella acanthifera*. All above-mentioned taxa are detritivorous and/or herbivorous amphipods, with only the genus *Caprella* containing species that can show strict omnivorous and carnivorous habits, while *P. longicaudata* show detritivorous and carnivorous habits (Supplementary Table S2).

Univariate GLM results showed that amphipod density and species richness significantly differed among areas (Table 1), specifically between RF and any other area (Supplementary Table S5). RF had higher total density and number of species (3023 ind. m^{-2}, 21 species) than ED (718 ind. m^{-2}, 5 species), GC (426 ind. m^{-2}, 11 species) and AL (316 ind. m^{-2}, 9 species) (Fig. 3).

3.2. Predictor variables influencing amphipod assemblages

Seagrass leaf area and epiphyte biomass were significant predictors determining variation in species abundances in amphipod assemblages, while their multivariate presence/absence was determined by seagrass density and epiphyte biomass (Table 2).

Seagrass leaf area and algal cover significantly influenced variation in the total density of amphipods (accounting for 76% of the variance), with amphipod density increasing with seagrass leaf area and algal cover (Table 3). Seagrass leaf area was the most important predictor of amphipod density according to the multi-model averaging, while it was the only relevant predictor causing an increase in amphipod species
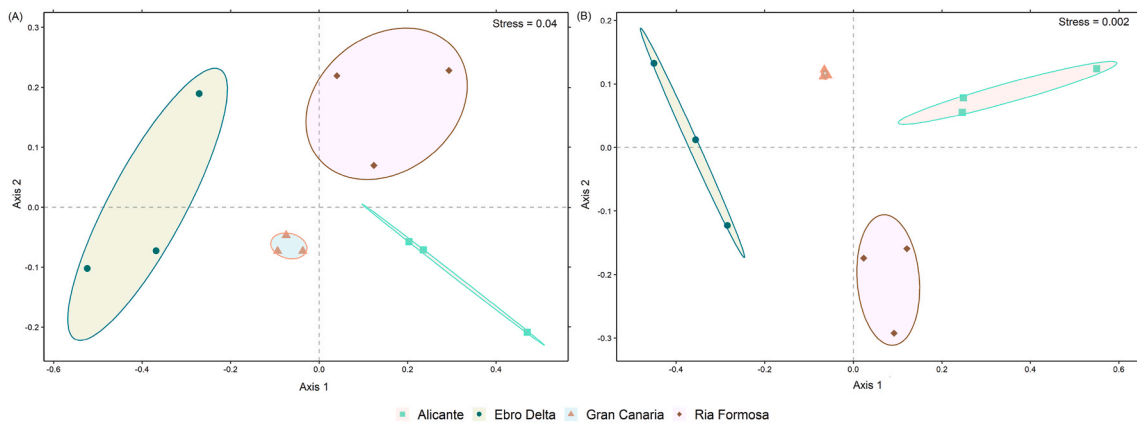
Fig. 2. Non-metric multidimensional scaling (nm-MDS) plot of meadows within each biogeographical area based on the species abundances (A) and presence/absence (B) of amphipod assemblages (Bray-Curtis and Jaccard distance, respectively).

Table 1
Summary of results of multivariate and univariate mixed GLMs examining variation in amphipod communities between biogeographical areas.

	Response variable	Fixed factor	Random factor	χ^2	df	P
Multivariate response	Species abundances	Area	–	–	3	**0.004**
	Species presence/absence	Area	–	–	3	**0.001**
Univariate response	Total density	Area	Meadow	11.6	4	**6.31e^{-09}**
	Species richness	Area	Meadow	9.4	4	**2.76e^{-07}**

richness (accounting for 83% of the total variance) (Table 4).

3.3. Patterns of variation in selected predictor variables

All directly selected predictors significantly varied among biogeographical areas (Supplementary Table S6). A significantly higher seagrass leaf area was found in RF than in the other biogeographical areas (Supplementary Table S7); however, the lowest leaf area was also found in a meadow from RF (Fig. 4A). Seagrass leaf area was not significantly correlated with any other variable (Supplementary Fig. S1 and Table S1). Seagrass density was significantly higher in ED than in any

other area (Fig. 4B, Supplementary Table S7). This predictor negatively correlated with the seagrass C: N ratio, while positively correlated with seagrass N content, latitude and sea temperature range (Supplementary Fig. S1 and Table S1). Algal cover was significantly higher in RF than in GC (Fig. 4C, Supplementary Table S7), with one meadow of RF (RF1) showing the highest algal cover. This predictor only significantly correlated with algal richness (Supplementary Fig. S1 and Table S1). Epiphyte biomass was significantly higher in RF than in ED and GC, with GC showing a significantly lower value than any other region (Fig. 4D, Supplementary Table S7)). This predictor negatively correlated with mean sea surface temperature, while positively correlated with sediment size and fine sediment.

Among indirectly selected predictors (i.e., those significantly correlated to directly selected ones) that significantly varied between biogeographical areas (Supplementary Table S6), seagrass C:N ratio was sequentially higher in GC than in AL followed by significantly lower values in RF and ED (Supplementary Fig. S2A and Table S7). Annual temperature ranges (Supplementary Fig. S2B and Table S8) monotonically vary with latitude (Supplementary Fig. S2C and Table S8), both showing higher values in AL and ED than in RF and GC. Lastly, fine sediment was significantly successively higher in RF followed by AL and then by ED, with GC showing significant lower values than RF but similar to AL and ED (Supplementary Fig. S2D, Table S7). Mean sea surface temperature, seagrass nitrogen, algal richness and sediment grain size did not differ significantly between areas (Supplementary Figs. S2E–H, Tables S6 and S7).
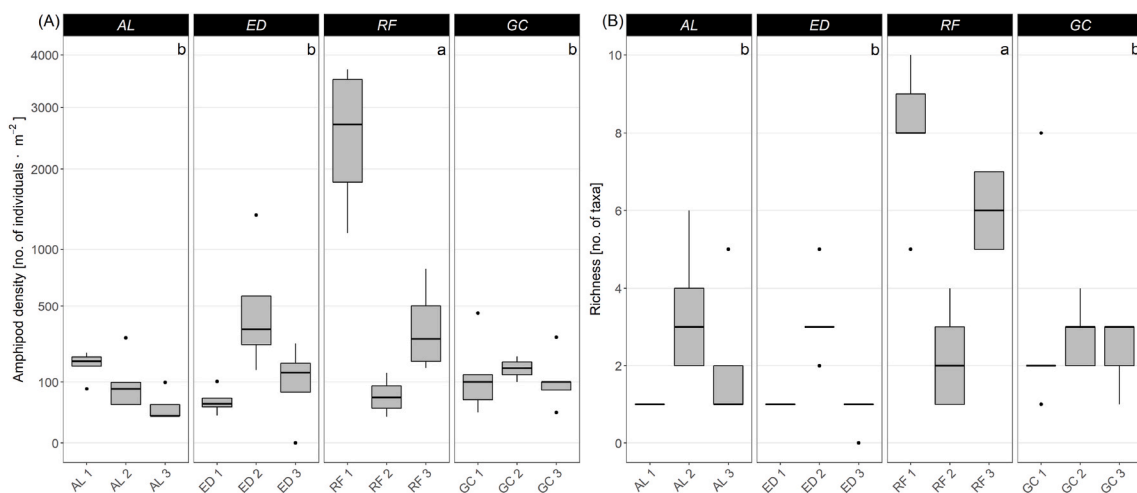

Fig. 3. Amphipod total density (A) and species richness (B) across meadows within the four biogeographical areas. Different lowercase letters denote statistically significant differences between areas based on mixed effect GLMs.

Table 2

Predictor variables determining patterns of variation in multivariate species abundances and species presence/absence in amphipod assemblages, according to results of the step-by-step model selection. The select model (i.e., the most parsimonious model with lowest AIC) shown in bold. (SST mean = Sea surface mean temperature).

Response variable	Model predictors	df	AIC
Species abundances	Seagrass leaf area	10	−4.72
	Algal cover	9	
	Seagrass density	8	
	Epiphyte biomass	7	
	Chlorophyll-a	6	
	Seagrass leaf area	10	−9.36
	Seagrass density	9	
	Seagrass leaf area	10	−11.77
	Seagrass leaf area	10	−12.22
	Algal cover	9	
	Seagrass leaf area	10	−15.56
	Chlorophyll-a	9	
	Seagrass leaf area	**10**	**−20.30**
	Epiphyte biomass	**9**	
Species presence/absence	Seagrass leaf area	10	−2.00
	Algal cover	9	
	Seagrass density	8	
	Epiphyte biomass	7	
	Chlorophyll-a	6	
	Seagrass density	10	−3.05
	Seagrass leaf area	9	
	Seagrass density	10	−3.10
	Seagrass density	10	−3.15
	Chlorophyll-a	9	
	Seagrass density	10	−3.19
	Algal cover	9	
	Seagrass density	**10**	**−4.97**
	Epiphyte biomass	**9**	

4. Discussion

Our results showed biogeographical differences in the abundance and presence/absence of amphipod species in assemblages, as well as in the total density and species richness of amphipods associated with *Cymodocea nodosa* meadows. These differences were mostly explained by variations in structural meadows attributes that enhance seagrass habitat (i.e., seagrass leaf area) and complexity (i.e., seagrass density, algal cover, and epiphyte biomass). Such attributes were concurrently influenced by differences in environmental conditions (i.e., nitrogen availability, latitude, temperature range and mean, sediment grain size and fine sediment) in the study areas across the Atlantic-Mediterranean

realm. This is consistent with the relatively large body of research that relates differences in assemblages of marine invertebrates in general, and amphipods in particular, with changes in structural attributes of the habitat in which they are found, with such attributes largely influencing the availability of space (Osman, 1977; Leite et al., 2007) and food (Edgar, 1990; Buzá-Jacobucci and Pereira-Leite, 2014), as well as refuge provision (Leber, 1985; Tuya et al., 2011) and predator-prey interactions (Orth et al., 1984; Alexander et al., 2012).

We found that the increase in habitat availability provided by seagrass leaf area was the most important determinant of biogeographic variation in species abundances in amphipod assemblages, as well as in their total density and species richness. Amphipod density increased with seagrass leaf area and algal cover (with both predictors explaining 76% of variation in density), while richness increased only with seagrass leaf area (83% of the variation explained). The positive relationship between amphipod total density or richness and macrophyte surface area is common not only for seagrass-associated amphipods, but also for amphipods associated with kelp hodlfasts (Tuya et al., 2011), macroalgae such as *Sargassum cymosum* (Leite et al., 2007) and *Caulerpa racemosa* (Vázquez-Luis et al., 2009), and even rhodoliths (Navarro-Mayoral et al., 2020). Particularly, Gartner et al. (2013) found that a higher seagrass surface area (i.e., in terms of surface area per shoot multiplied by shoot density) and algal epiphytes, experimentally manipulated using artificial mimics, favoured habitat selection by amphipods, thus increasing their density.

In our study and in general, *C. nodosa* act as primary foundation species and habitat facilitator (*sensu* in Thomsen et al., 2010 and 2018), providing the physical space for the settlement of secondary species, such as algal epiphytes or macroalgae entangled around seagrass leaves. In this way, Knowles and Bell (1998) found that the total density of epifaunal communities dominated by amphipods was higher on drift macroalgae than on seagrasses with contrasting architectures, thus suggesting a stronger influence of macroalgae found in the meadow than of plant biomass. Drifting macroalgae contributes to increase habitat complexity in seagrass meadows, by providing temporary habitats with micro-refuges and a wide range of additional nutrient-rich food resources for epifaunal macroinvertebrates (Rodil et al., 2021), and particularly for amphipods (Leber, 1985; Jernakoff and Nielsen, 1997; Cook et al., 2011; Myers and Heck, 2013). Most algae have a high nutritional value, greatly exceeding that of their host seagrasses, and are more digestible for amphipods (Hyndes and Lavery, 2005), as also occurs with algal and seagrass detritus (Zimmerman et al., 1979). Furthermore, high algal cover and seagrass leaf area may increase refuge provision against predators, which are known to exert an important
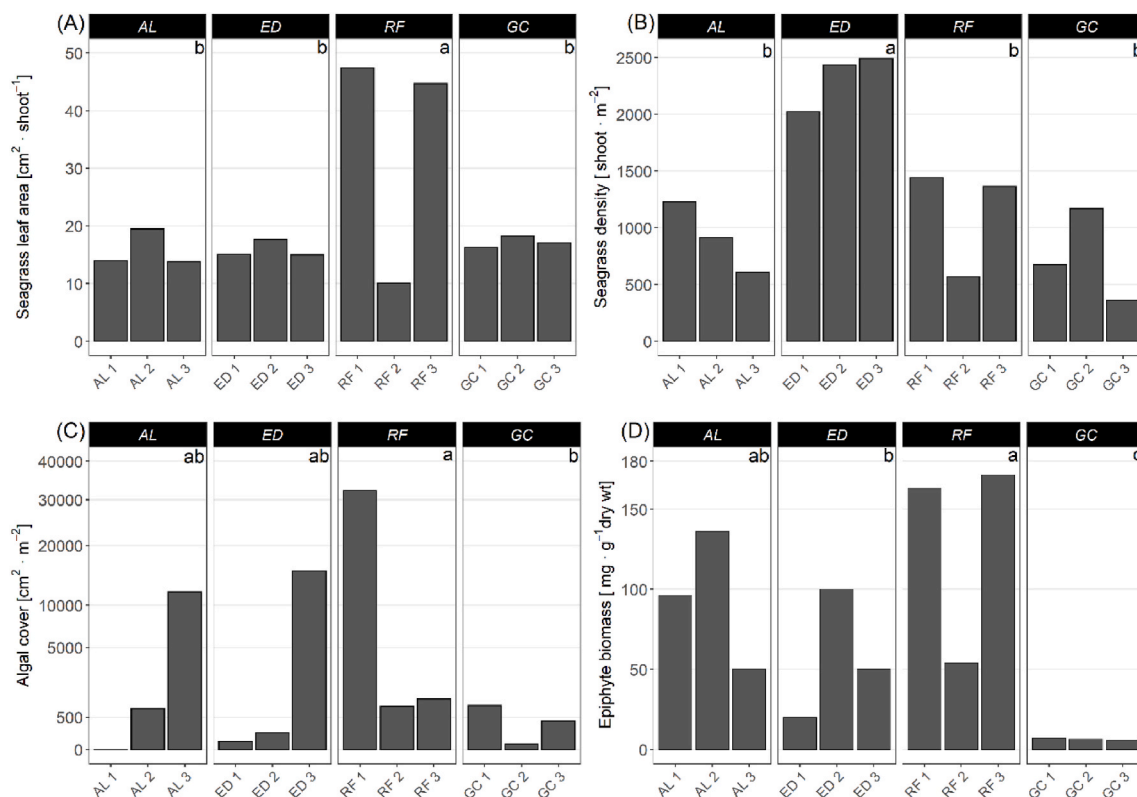
Table 3

Predictor variables determining patterns of variation in total density and species richness of amphipods according to results of univariate model selection from stepwise approach and multimodel averaging (both approaches providing same results here). The Breusch-Pagan heteroskedasticity tests and collinearity diagnosis (via de VIF) among predictors (when more than one was selected) are included. See full results in Supplementary Table S9..

Response variable	Stepwise selection	Multimodel averaging selection					
	Significant predictors (*p*-value)	Model predictors (VIF)	df	AICc	weight (wi)	Adjusted R^2	Breusch-Pagan test (*P*)
Total density	Seagrass leaf area (*p* = 0.03), algal cover (*p* = 0.009)	Seagrass leaf area (VIF = 1.39), algal cover (VIF = 1.38)	4	184.9	0.89	0.76	BP = 3.27 (*p* = 0.19)
Species richness	Seagrass leaf area (*p* = 2.04 e^{-05})	Seagrass leaf area	3	37.3	0.62	0.83	BP = 0.09 (*p* = 0.75)

Table 4

Relative importance and estimates of predictor variables determining the total density and species richness of amphipods from multimodel averaging of GLMs (full method: sum of Akaike weights over all possible models) using the 'MuMIn' package. Significant predictors are highlighted in bold.

Response variable	Predictor	Estimate	Adjusted SE	z statistic	*P*	Relative importance
Total density	Seagrass leaf area	6.85	1.94 e^{-02}	3.52	**0.0004**	1.00
	Algal cover	4.30	2.37 e^{-05}	0.55	**0.04**	0.89
Species richness	Seagrass leaf area	0.16	0.02	7.50	**2.04e^{-05}**	1.00

Fig. 4. Predictor variables selected by GLM as main determinants of the variation in the total density of amphipods (A, C) and in the species richness (A), as well as in the species abundances (A, D) and presence/absence (B, D). Bars are mean values across meadows within the four biogeographical areas Different lowercase letters denote statistically significant differences between areas based on GLM results.

control on amphipod populations, reducing amphipod biomass by more than 90% when the algal cover in seagrass meaows is low (Moksnes et al., 2008; Reynolds et al., 2018). Overall, algal cover may promote bloomed densities of herbivorous and detritivorous amphipods, which were dominant in our study, by increasing the availability of high-quality food. Furthermore, drifting macroalgae also contribute to species dispersion, thus increasing connectivity with surrounding habitats (Salovius et al., 2005).

We found that seagrass leaf area and algal cover were much higher in RF, where amphipods were notably more abundant and richer in number of species than in any other area. However, a RF meadow (RF2) had the smallest seagrass leaf area, due to intense fish herbivory on seagrass shoots (Martínez-Crego et al., 2021), which resulted in a lower amphipod density, with a similar value than that found in the other three areas. One meadow from RF (RF1) presented the highest cover and richness of algae recorded in our study, which resulted in the highest amphipod density (a total of 2594 ind. m^{-2}). Of the total number of individuals in this meadow, 74% corresponds to *Gammarella fucicola*, a detritivorous species (Guerra-García et al., 2014), with the potential to actively feed on seagrass (*Posidonia oceanica*) leaves and seeds (Castejón-Silvo et al., 2019) but preferentially assimilating carbon from seagrass epiphytes and drift macroalgae (Lepoint et al., 2006).

In our study, species abundances in amphipod assemblages only significantly differed between ED and AL, although the main drivers of variation in species abundances (i.e., seagrass leaf area and epiphyte biomass) were similar in both biogeographical areas. These two areas also showed similar values for indirectly selected predictors of species abundances, such as sea mean temperature and sediment grain size, while the proportion of fine sediment was higher in AL than in ED. This result contrasts with the higher sedimentation and proportion of fine sediment typically reported in enclosed than in open systems (Franz and Tanacredi, 1992; Gil et al., 2006). However, it could be explained by the

deposit of larger sediment reported in the enclosed ED area associated to interannual variation in circulation regimes, land input through river floods and storms (Guillén and Jimenez, 1995; Guillén et al., 2005; Besset et al., 2019). The higher proportion of fine sediment in AL is likely driving the selection of *C. dellavallei* as the only indicator species in this area, in terms of both, species abundances and presence/absence, since this species has been previously reported as present only in bottoms with fine sediment compared to larger grain sizes (Carvalho et al., 2012). Regarding other species exclusive to this area, but present only in one or two meadows (i.e., not significant due to fidelity <1), previous studies have reported *A. brevicornis* presence (Robertson et al., 1989) and *A. diadema* high abundances (Ruffo, 1982) as associated to a high proportion of fine sediment or to muddy bottoms. This is likely required for a successful feeding in such species (Massé, 1971). Overall, grain size has been reported as an important determinant of the presence of certain species and the absence of others in amphipod assemblages (Fincham, 1973; Parker, 1984).

Our results revealed that amphipod assemblages differed between biogeographical areas more in terms of species presence/absence than in terms of abundances, suggesting a prevalent role of rare species in assemblage's turnover that agrees with previous studies (Costello and Myers, 1996). While the abundances only showed differences between only two areas (ED and AL), the species presence/absence differed between more areas. Indeed, we found that species present in GC significantly differed from those in any other area. Such pattern of variation was largely driven by seagrass density and epiphyte biomass, together with covarying seagrass N and C:N, latitude, fine sediment, and sea temperature mean and range. The lowest biomass of seagrass epiphytes and the highest seagrass C:N ratio was found in GC, with meadows in this subtropical area also showing a significantly lower seagrass density than ED, and lower latitude and sea temperature range than AL and ED. The only indicator species found in GC, *P. longicaudata,* is an

opportunistic amphipod with a tube-dwelling lifestyle and a detritivorous feeding strategy (Flynn and Valério-Berardo, 2009; Guerra-García et al., 2014). Thus, the presence of this species is likely unaffected by the lowest epiphyte biomass in GC in terms of either refuge against predators or availability of food directly consumed.

In contrast, indicator amphipods of RF (Aoridae n.i, *Iphimedia* sp. and *Caprella acanthifera*) included detritrivorous but also herbivorous species (Guerra-García et al., 2004 and 2014; Michel et al., 2015; Rumbold et al., 2018), whose presence could be favoured by a larger epiphyte biomass in terms of food supply. *C. acanthifera* and members of Aoridae family (e.g., *Aora spinicornis)* are reported as active consumers of *P. oceanica* epiphytes (Michel et al., 2015). Also, *Iphimedia* sp. and *C. acanthifera* and some Aoridae species are mobile non-tube-building amphipods, for which a mixed diet including macroalgae and epiphytes has been reported as an efficient strategy (Cruz-Rivera and Hay, 2000). Similarly, the higher seagrass nutritional quality (i.e., lower seagrass C:N ratio with a reduced "nitrogen dilution" effect) found in RF compared to GC may favoured the presence of herbivorous amphipods able to feed on the seagrass. For such kind of mesograzers, higher consumption rates are reported in low C:N seagrass often associated with concurrently lower structural (e.g., leaf breaking force and thickness) and chemical (e.g., lower accumulation of phenolic compounds) defences (Martínez-Crego et al., 2016). Furthermore, high proportion of fine sediments (such as that found in RF) are typically associated to low hydrodynamic conditions (de Boer, 2007; Cabaço et al., 2010), which favour the occurrence of the indicator species C. acanthifera (Krapp-Schickel and Vader, 1998; Lolas and Vafidis, 2013; Guerra-García, 2001). In general, different species within the Caprellidae family are usually found in environments exposed and protected from wave action (Guerra-García, 2001) or depth-related currents (Jacobucci et al., 2019). Low hydrodynamics enhance sedimentation rates and the accumulation of organic matter, thus favouring food availability for detritivorous amphipods (Conradi et al., 1997). No influence of sea temperature mean is expected in our study, since variations in mean temperature were minimal (ca. 2 °C) and not significant between areas, even not reaching the minimum variation (5–10 °C) reported to cause changes in amphipod metabolic rates (Maranhão and Marques, 2003).

We found that the species shared between areas were minority (2 shared species of 5 in ED; 4 of 9 in AL; 7 of 21 in RF and 2 of 11 in GC), although not all the amphipod taxa that appeared exclusively in one area were significantly representative of it. This was the case of the three taxa (i.e., Ischyroceridae n.i, *G. aequicauda*, and *Corophium* sp.) exclusively found only in ED, with no significant indicator value either in term of abundance or presence. However, their presence exclusively in ED is consistent with their life patterns, since the euryhaline species *Gammarus aequicauda* is characteristic and abundant in ED (Delgado et al., 2011), while the genus Corophium is characteristic of high energy channels with organic-poor sediments in sheltered bays and estuaries (Parker, 1984). Seagrass density was significantly higher in ED than in any other area. Thus, the decline in amphipod predation under a higher shoot density canopy (Reynolds et al., 2018), together with a higher production of higher-quality seagrass detritus in more dense meadows with lower C:N (i.e., higher N availability), are likely contributing to promote ED meadows as suitable habitats for several amphipod taxa with no clear indicator value. In our study, annual temperature ranges monotonically vary with latitude, contributing to explain habitat suitability for certain indicator species in AL and ED, which had a higher latitude (i.e., temperate vs. subtropical) and sea temperature range compared to GC. Amphipod turnover (i.e., colonization, growth, reproduction, and survival) in temperate regions is commonly adjusted to seasonal variation in environmental conditions (e.g. temperature, photoperiod), food resources (e.g. macrophyte quality and biomass for herbivorous amphipods) and energetic requirements (Neuparth et al., 2002; Maranhão and Marques, 2003). Among amphipod species specific to the subtropical GC with no significant indicator value, the caprelid amphipod *Caprella caveidinae* could be endemic to this area (Riera et al.,

2003). In GC, the island effect may contribute to promote amphipod richness and endemicity (Arfianti and Costello, 2020).

We found that seagrass density covaried with N availability, latitude and temperature range. The co-variation of seagrass density with nutrient availability is coherent with results of N enrichment experiments (Bulthuis et al., 1992), although excess nutrients may promote epiphyte and algal blooms that negatively affect seagrass survival, and subsequently seagrass density (Martínez-Crego et al., 2014; Lee et al., 2007; Schmidt et al., 2012). Similarly, the observed increase in nitrogen availability (i.e., lowered plant C:N) with latitude is consistent with previous studies in terrestrial and aquatic plants (Borer et al., 2013; Reich and Oleksyn, 2004). We also detected that epiphyte biomass co-varied with mean sea temperature, sediment grain size and proportion of fine sediments.

5. Conclusions

Our results highlight the importance of *Cymodocea nodosa* meadows and associated drifting macroalgae to support amphipod assemblages, which fulfil key ecological functions such as the transfer of energy to higher trophic levels or recycling matter. We evidenced that biogeographical patterns of variation in the amphipod community were mostly determined by structural attributes of the meadow that increase habitat availability (i.e., seagrass leaf area) and complexity (i.e., seagrass density and algal cover). Such attributes covary with environmental conditions (i.e., nutrient availability, proportion of fine sediments, latitude or sea temperature range) to determine amphipod patterns of variation. Overall, the understanding of the interplay of drivers acting at geographic and local sales is revealed as critical for a clear prediction of variation in amphipod communities across biogeographical areas.

CRediT authorship contribution statement

Sandra Navarro-Mayoral: Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Fernando Tuya:** Conceptualization, Methodology, Sample collection, Formal analysis, Supervision, Writing – review & editing. **Patricia Prado:** Methodology, Sample collection, Writing - review & editing. **Candela Marco-Méndez:** Methodology, Sample collection, Writing - review & editing. **Victoria Fernandez-Gonzalez:** Writing - review & editing. **Yolanda Fernández-Torquemada:** Sample collection, Writing – review & editing. **Fernando Espino:** Sample collection, Writing - review & editing. **Jose Antonio de la Ossa:** Taxonomy work. **David Mateu Vilella:** Taxonomy work. **Margarida Machado:** Taxonomy work, Writing - review & editing. **Begoña Martínez-Crego:** Conceptualization, Methodology, Sample collection, Data curation, Formal analysis, Funding acquisition, Supervision, Writing - review & editing.

Declaration of competing interest

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

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2023.105918.

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