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## Diversity, community structure and habitat use of molluscs in marinas from the Iberian Peninsula and Northern Africa



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#### ABSTRACT

Although the number of marinas along shorelines has significantly increased during the last years, there is a lack of studies on mobile epifauna associated to floating pontoons or infauna inhabiting soft bottoms of marinas. Artificial hard substrate (lateral surface of pontoons) and soft substrate (sediment) were sampled in 42 marinas along the Iberian Peninsula and Northern Africa and 48 different species of molluscs were found. Species richness and Shannon diversity values were higher in sediments than in hard substrates; multivariate analyses also revealed differences in species composition and trophic guilds between these two habitats. The most abundant species on the hard substrate were *Mytilus galloprovincialis* and *Musculus subpictus*, and *Corbula gibba* and *Parvicardium exiguum* in sediments. Although filter feeders were dominant in both habitats, herbivorous species were only found on the hard substrate while deposit feeders were restricted to sediments. As diversity, abundance and trophic patterns were not always consistent for all localities, a local scale effect must be considered in environmental programs for marinas. None exotic mollusc was detected, so this study provides baseline information necessary as a first step in monitoring programmes for early detection of new incoming exotic species. Faunistic knowledge is mandatory to provide local governments, policy makers and authorities with readily accessible species datasets and ecological information. This should be a priority to address an integrated and sustainable management of marinas.

## 1. Introduction

Coastlines are being increasingly urbanized (Firth et al., 2016) and, therefore, artificial structures are becoming dominant (Kenworthy et al., 2018). The important inflow of the population to coastal areas is leading to an increment in recreational boating (Davenport and Davenport 2006; Gómez et al., 2017). Although marinas are important drivers of socio-economic progress (Gómez et al., 2019; Valdor et al., 2019), their construction requires previously dredging and shoreline straightening, which destroys the original habitat (Iannuzzi et al., 1996).

Boating activities in marinas introduce a range of environmental problems, such as emission of air pollutants, noise, sediment dredging, wastewater discharges, oil spills, leaks of petroleum derivatives (such as hydrocarbons), and accumulation of heavy metals and biocides from antifouling paints (McGee et al., 1995; Mali et al., 2017; Toh et al., 2017). This has a relevant impact on water quality and sediments

(Guerra-García and García-Gómez 2005a,b; Petrosillo et al., 2010; Sim et al., 2015; Gómez et al., 2017, 2019; Guerra-García et al., 2021a). Furthermore, marinas have suitable conditions for establishment and spread of fouling organisms and are presently regarded as important hotspots for Non Indigenous Species (NIS) (Ros et al., 2013a; Ashton et al., 2014; Foster et al., 2016; Ferrario et al., 2017; Kenworthy et al., 2018; Martínez-Laiz et al. 2018, 2019; Ulman et al., 2019a).

Despite the importance of marinas as chemical and biological pollution sinks, the knowledge about these systems is very scarce. Although some research has been carried out to evaluate the pollution pressure (Martínez-Lladó et al., 2007; Mali et al., 2017; Gómez et al., 2019), very little efforts have been conducted to properly characterize the species composition of their flora and fauna (Covazzi Harriague et al., 2012; Chatzinikolaou et al., 2018). Marinas provide a wide and extensive range of habitats, including soft bottoms below (normally muddy sediments) and hard surfaces for attachment on pontoons (and

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associated ropes and buoys), breakwaters and pilings. Berthing pontoons, one of the most ubiquitous structures in marinas, are known to provide recruitment substrate for marine biota but the ability of pontoons to support marine life in modified areas has not been comprehensively studied (Toh et al., 2017). Knowledge on the distribution of epibiotic assemblages on pontoons is essential to identify the factors that contribute to spatial variation and encourage the design and construction of ecologically-friendly marinas (Toh et al., 2017). Basic knowledge of benthic communities inhabiting marinas (e.g. Moreno et al., 2009; Chatzinikolaou et al., 2018; Gavira O'Neill et al., 2018; Ng et al., 2019) is mandatory to understand ecological functioning in these recreational harbours. Therefore, an integrative approach including lists of species and ecological information is pivotal as baseline to properly address a sustainable management of marinas.

Available faunistic information in marinas is mainly restricted to sessile communities and/or NIS (e.g. Di Franco et al., 2011; Oricchio et al., 2016; Kenworthy et al., 2018; Ulman et al., 2019b; Ferrairo et al., 2020) and there is a lack of studies targeting mobile epifauna from floating pontoons and from soft bottoms of marinas. Crustaceans, molluscs and polychaetes are the dominant groups inhabiting marinas (Guerra-García and García-Gómez 2005a; Fernández-Romero et al., 2017). Most of studies have dealt with crustaceans (focused especially on peracarids, since they include a remarkable number of NIS, see e.g. Ros et al., 2013a; Marchini et al., 2015; Martínez-Laiz et al., 2018) or polychaetes (Fernández-Romero et al., 2019), and comprehensive mollusc surveys are still lacking. Molluscs are one of the dominant groups of the marine communities and they contribute largely to the local biodiversity (Rueda et al., 2009; Rubal et al., 2018), making up as much as 25% of the species in the marine benthos (Appeltans et al., 2012). A total of 2466 marine Mollusca species have been reported from Spanish waters, representing around 5.5% of the worldwide marine molluscan richness (Gofás et al., 2017). Molluscs, both epifaunal communities on seaweeds and those inhabiting sediments, have been successfully used as bioindicators of coastal environmental conditions in the Iberian Peninsula (Sánchez-Moyano et al., 2000; Guerra-García and García Gómez 2004; Moreira et al. 2005, 2010; Moreira et al. 2005, 2010). Due to their economic and ecological importance, as well as sedentary life, molluscs have assumed a major role in monitoring contaminants (Boening 1999; Feldstein et al., 2003). Moreover, some molluscs are important nutrititious food source or have a great health-promoting potential in Marine Biotechnology through their antiviral, anti-inflammatory and antimicrobial properties (Khan and Liu 2019).

Regarding molluscs from marinas, there is a lack of studies characterizing assemblages associated to artificial hard substrate of pontoons (Tempesti et al., 2020) and most of the available information is derived from studies of the sediment (Covazzi Harriague et al., 2012; Chatzinikolaou et al., 2018; Ng et al., 2019). Furthermore, in most cases, information about molluscs in sediment from marinas is fragmentary since it is integrated in more general studies encompassing large ports, estuaries, etc. (e.g. Estacio et al., 1997; Guerra-García and García-Gómez 2004; Moreira et al., 2005). To fill this gap, the main objectives of the present study are (i) to describe mollusc assemblages from marinas of the Iberian Peninsula and Northern Africa and (ii) to explore potential differences between mollusc community structure and trophic guilds in artificial hard substrate (lateral surface of pontoons) (hereinafter AHS) and soft substrate (sediment) (hereinafter SS). This baseline information is necessary as a first step in monitoring programmes to early detect potential NIS, to alert about pollution sources, and ultimately to contribute with basic tools for assessing a comprehensive and sustainable management of marinas.

#### 2. Material and methods

## 2.1. Sampling surveys and laboratory processing

A first exploratory study was conducted along the whole Iberian Peninsula and North Africa (Fig. 1). Pontoons of 88 marinas were surveyed from May to June 2011 (late spring and early summer) as part of a general sampling program to characterize the expansion of the amphipod Caprella scaura Templeton (1836) throughout the Iberian Peninsula and adjacent areas (see Ros et al., 2014). To characterize epibiotic mollusc assemblages, sampling efforts were focused in a well-defined secondary substrate, Bugula neritina (Linnaeus, 1758), which grows on the AHS of the lateral surface of pontoons (primary substrate). This bryozoan is among the dominant basibionts of fouling communities in harbours and marinas of the Iberian Peninsula, and it hosts a diverse epifaunal community (see e.g. Conradi et al., 2000; Ros et al., 2015; Fernández-Romero et al., 2019). B. neritina was present in 42 of the 88 marinas. In each of those 42 marinas, three colonies of B. neritina were collected from the lateral submerged area of pontoons, close to the water surface. Each colony was directly removed by hand, kept in a plastic container and preserved in 96% ethanol.

Taking into account that the number of species and total abundances of molluscs associated to *B. neritina* in the first exploratory study were rather low, a second additional study using global scrapes instead of a single arborescent substrate was conducted in June–July 2017 based on an experimental design to test for habitat differences among marinas



**Fig. 1.** Study area showing the location of the 88 marinas surveyed. White circles indicate marinas were *Bugula neritina* were absent, while black circles (1–42) indicate the marinas where *B. neritina* were present and it was collected for the general study of molluscs along the Iberian Peninsula and Northern Africa. The six marinas selected for the comparative study of mollusc assemblages in artificial hard substrate (AHS) vs soft substrate (SS) are indicated (Chi: Chipiona, Ame: Puerto América, Bar: Barbate, Lin: La Línea, Fue: Fuengirola, Alm: Almería).

along a Mediterranean-Atlantic axis. Six marinas were selected in Southern Iberian Peninsula, three in the Atlantic coast (Chipiona, Puerto América and Barbate) and three in the Mediterranean (La Línea-Puerto Chico, Fuengirola and Almería) (Fig. 1). Detailed environmental data of these marinas are included in Guerra-García et al. (2021a). Within each marina, two habitats were considered: AHS and SS. Three floating pontoons were randomly selected at each marina. At each pontoon, three random quadrats (15  $\times$  15 cm) were located on the lateral submerged surface (approximately 10 cm below water level) and all taxa inside each quadrat (secondary substrates) were scraped and fixed in 96% ethanol. Additionally, at each pontoon three sediment samples were collected from the bottom using a small van Veen grab (area 15  $\times$ 15 cm). The depth of the bottom at the marinas (where the sediment was collected) ranged from 3.5 to 6 m (3.5 m in Barbate and La Línea, 4 m in Chipiona and Almería, 5 m in Fuengirola, and 6 m in Puerto América). Samples were washed through a sieve with a mesh size of 0.5 mm and the retained fraction was fixed in 96% ethanol stained with Rose Bengal.

In the laboratory, molluscs were sorted, identified to species level when possible, and counted.

The molluscan species were characterized in terms of their feeding guilds according to Moreira (2003), Rueda (2007) and Rueda et al. (2009). The following categories were considered: *filter feeders* (F), capturing the seston particles with their gills and/or with mucous strings; *herbivores* (H), feeding on micro and macro algae; *deposit feeders* (D), feeding on organic particles contained in the sediments or water column; *carnivores* (C), feeding on other organisms, such as molluscs or polychaetes; *scavengers* (S), feeding on remains of dead organisms; *ectoparasites* and specialized carnivores (E), feeding on much larger organisms without mobility on which they live during their life cycle.

## 2.2. Statistical analyses

The experimental design to compare the molluscan assemblages in marinas of Southern Spain included four factors: 'Location' (Lo), a fixed factor with two levels (Mediterranean vs Atlantic); 'Marina' [Ma (Lo)], a random factor with three levels nested with Lo; 'Pontoon' [Po(Ma (Lo))], a random factor nested with Ma and Lo; 'Habitat' (Ha), a fixed factor with two levels (AHS vs SS). The Atlantic marinas were Chipiona (Chi), Puerto América (Ame) and Barbate (Bar), and the Mediterranean ones were La Línea (Lin), Fuengirola (Fue) and Almería (Alm). The number of replicates was n = 3, corresponding with the three van Veen grabs or the three scraped quadrats at each pontoon. For each replicate, number of mollusc species (S), total abundance (individuals/225 cm<sup>2</sup>) and Shannon-Wiener's Diversity (H') were calculated. To compare these community descriptive parameters, a four-way ANOVA was conducted. Homogeneity of variances was checked using Cochran's test. When ANOVA detected significant differences for a given factor or interaction of factors, the source of the difference was identified by applying the Student-Newman-Keuls (SNK) test (Underwood 1997). Non-parametric multidimensional scaling (MDS) was carried out to show the relationship among locations (Atlantic vs Mediterranean), habitats (AHS vs SS) and marinas, according to the mollusc assemblages (based on species composition and their abundance). Differences in the mollusc assemblage among locations, marinas, pontoons and habitats were tested by a permutational multivariate analysis of variance (PERMANOVA), using the same experimental design explained above. Analysis was based on Bray-Curtis similarities and Monte Carlo tests were included. Significant P-values were obtained by computing 9999 permutations of residuals under a reduced model, as this method gives the most accurate Type I error for complex design (Anderson 2005). Pairwise comparisons were then used. To explore differences at the trophic level, an additional PERMANOVA using the same experimental design was conducted using abundances of filter feeders, herbivores, deposit feeders, carnivores, scavengers and ectoparasites. An additional MDS was carried out to show the relationship among locations (Atlantic vs Mediterranean), habitats (AHS vs SS) and marinas based on abundance of each feeding group. PERMANOVA analyses were carried out using PRIMER v.6+PERMANOVA package (Clarke and Gorley 2006) and ANOVA was conducted on GMAV5 software (Underwood et al., 2002).

## 3. Results

1852 individuals belonging to 48 different species of molluscs were found at marinas during the present study.

Regarding the first exploratory study along the whole Iberian Peninsula and Northern Africa, 11 species (641 specimens) were found inhabiting *B. neritina*. The dominant species was *Mytilus galloprovincialis*, with about 93% of total abundance (Table 1).

Forty-two species were collected in the second study focused on the six marinas of the Southern Iberian Peninsula, corresponding to 11 species (688 specimens) on AHS and 32 species (509 specimens) in SS. For the AHS, the pattern was similar to that one obtained for the bryozoan along the Iberian Peninsula: a clear numerical dominance of

## Table 1

Total number of mollusc specimens collected from the bryozoans *Bugula neritina* in the 42 marinas sampled along the Iberian Peninsula and Northern Africa. Feeding guild (FG) according to Moreira (2003), Rueda (2007) and Rueda et al. (2009) are included for each species (F: Filter feeders, H: Herbivores, E: Ectoparasites).

		Bugula neritina				_						
			Biva	lves				Gas	strop	ods		
	FG	F	F	F	F	Н	Е	н	н	н	н	
		Hiatella arctica	Mytilus galloprovincialis	Musculus subpictus	Sphenia binghami	Barleeia unifasciata	Doto sp.	Pusillina inconspicua	Rissoa similis	Rissoa parva	Setia sp.	Tergipedidae undet.
<ol> <li>Santander (43.45° N, 3.82° W)</li> <li>Gijón (43.54° N, 5.67° W)</li> <li>A Graňa (43.48° N, 8.26° W)</li> <li>A Coruña (43.37° N, 8.40° W)</li> <li>Nazaré (39.59° N, 9.07° W)</li> <li>Cascais (38.69° N, 9.42° W)</li> <li>Sines (37.95° N, 8.87°)</li> <li>Albufeira (37.08° N, 8.27° W)</li> <li>Faro (37.01° N, 7.94° W)</li> </ol>			• • • • • • •	•	•	•	•	•		•		•
<ol> <li>Isla Cristina (37.19° N, 7.34° W)</li> <li>El Rompido (37.22° N, 7.13° W)</li> <li>Chipiona (36.74° N, 6.43° W)</li> <li>Rota (36.62° N, 6.35° W)</li> <li>Potro América (36.54° N, 6.38° W)</li> <li>Sancti Petri (36.40° N, 6.21° W)</li> </ol>					•							
<ol> <li>Conil (36.29° N, 6.14° W)</li> <li>Barbate (36.19° N, 5.93° W)</li> <li>Tánger (35.79° N, 5.81° W)</li> <li>Ceuta (35.89° N, 5.32° W)</li> <li>Ceuta (35.89° N, 5.32° W)</li> <li>Marina Smir (35.75° N, 5.34° W)</li> <li>M'Dia (35.68° N, 5.31° W)</li> </ol>												
22. La Linea (36.16° N, 5.36° W) 23. Fuengirola (36.54° N, 4.62° W) 24. Benalmádena (36.60° N, 4.51° W) 25. Málaga (36.72° N, 4.41° W) 26. La Caleta (36.75° N, 4.07° W)			•								•	
27, Mottil (36, 72° N, 3, 53° W) 28, Almerimar (36, 70° N, 2, 79° W) 29, Roquetas de Mar (36, 76° N, 2, 61° W 30, Almería (36, 83° N, 2, 46° W) 31, Carbonera (36, 99° N, 1, 90° W) 32, Torrevieja (37, 97° N, 0, 68° W)	/)	•	•									
33. Alicante (38.34° N, 0.49° W) 34. Dénia (38.85° N, 0.11° W) 35. Valencia (39.43° N, 0.33° W) 36. Borriana (39.86° N, 0.07° W) 37. Oropesa (40.08° N, 0.13° E)			• • •									
88. Benicarló (40.42° N, 0.43° E) 99. Tarragona (41.11° N, 1.25° E) 40. Vilanova (41.21° N, 1.73° E) 41. Barcelona (41.38° N, 2.18° E) 42. L'Estartit (42.05° N, 3.21° E)			•						•			
otal number of specimens:	• 1-1	0 ir	nd	•	11-	100	ind		10	)1-1	000	) ind

T

*M. galloprovincialis* (92% of total abundance) followed by *M. subpictus* (3%) (Table 2). Samples obtained by scraping of the quadrats, however, allowed for the collection of some species from the surface of the floating pontoons which were not found associated to the arborescent bryozoan (i.e. the bivalves *Ostraea stentina* and *Parvicardium scriptum*, the gastropods *Haminea* sp., *Runcina* sp. and *Setia amabilis*, and the

polyplacophoran *Acanthochitona crinita*). For the SS, the dominant species corresponded to bivalves, i. e *Corbula gibba* (26% of total abundance), *P. exiguum* (14%), *Abra nitida* (10%) and *A. tenuis* (8%) (Table 2).

The number of mollusc species (S) was higher in SS in comparison with AHS, in most of the marinas (Fig. 2, see SNK tests for the interaction Ha x Ma (Lo) in Table 3) and no significant differences were found

#### Table 2

Mollusc abundance (ind/ $m^2$ ) in artificial hard substrate (AHS) and soft substrate (SS) of the six selected marinas in Southern Spain. Feeding guild (FG) according to Moreira (2003), Rueda (2007) and Rueda et al. (2009) is included for each species (D: Deposit feeders, F: Filter feeders, C: Carnivores, H: Herbivores, S: Scavengers, E: Ectoparasites).





Fig. 2. Number of mollusc species, total abundance and Shannon-Wiener Diversity (H') values in each habitat (AHS, SS) at each marina. Values are expressed as mean  $\pm$  SE. Chi: Chipiona, Ame: Puerto América, Bar: Barbate, Lin: La Línea, Fue: Fuengirola, Alm: Almería. Significant differences are indicated in Table 3.

## Table 3

Summary of the four-way ANOVA results for number of species, total abundance and Shannon diversity of mollusc assemblages. Lo: Location (Atl: Atlantic, Med: Mediterranean); Ma (Lo): Marina nested with location (Chi: Chipiona, Ame: Puerto América, Lin: La Línea, Bar: Barbate, Fue: Fuengirola, Alm: Almería); Po(Ma (Lo): Pontoon nested with marina and location; Ha: Habitat (AHS: artificial hard substrate, SS: soft substrate). df: degrees of freedom; MS mean squares; P: level of significance: \*P < 0.05, \*\*P < 0.01, n.s. not significant. See Fig. 2 for graphical representation of interactions Ha x Ma (Lo).

Source of variation	df	MS	F	Р	MS	F	Р	MS	F	Р
		Number of Sp	pecies (S)		Total Abund	lance (N)		Shannon Di	versity (H')	
Lo	1	7.7870	0.33	0.5982 n.s.	2.0754	0.56	0.4960 n.s.	0.5391	0.48	0.5253 n.s.
Ma (Lo)	4	23.8426	9.94	0.0009***	3.7080	3.78	0.0325*	1.1162	9.73	0.0010**
Po(Ma (Lo))	12	2.3981	1.80	0.0645 n.s.	0.9802	1.53	0.1338 n.s.	0.1148	0.70	0.7452 n.s.
На	1	126.7500	6.04	0.0699 n.s.	8.6198	3.59	0.1308 n.s.	11.2709	12.33	0.0246*
Lo x Ha	1	46.6759	2.22	0.2102 n.s.	16.4977	19.72	0.0113*	0.6110	0.67	0.4596 n.s.
Ha x Ma (Lo)	4	20.9907	11.06	0.0005***	2.3978	2.83	0.0726 n.s.	0.9143	4.67	0.0166*
Ha x Po(Ma (Lo))	12	1.8981	1.42	0.1754 n.s.	0.8469	1.32	0.2259 n.s.	0.1957	1.20	0.3029 n.s.
Residual	72	1.3333			0.3512			0.1278		
Total	107									
Cochran's test		C = 0.1111 n	1.S.		$C = 0.922 \ P < 0.01$		C = 0.1465  n.s.			
Transformation		None			Ln (x+1)			None		
SNK tests:										
Lo x Ha		Atl:	AHS = SS		Atl:	AHS = SS		Atl:	AHS = SS	
		Med:	AHS = SS		Med:	AHS > SS		Med:	AHS = SS	
Ha x Ma (Lo)		Chi:	AHS < SS		Chi:	AHS = SS		Chi:	AHS = SS	
		Ame:	AHS < SS		Ame:	AHS = SS		Ame:	AHS < SS	
		Bar:	AHS < SS		Bar:	AHS = SS		Bar:	AHS < SS	
		Lin:	AHS = SS		Lin:	AHS = SS		Lin:	AHS < SS	
		Fue:	AHS = SS		Fue:	AHS > SS		Fue:	AHS = SS	
		Alm:	AHS < SS		Alm:	AHS = SS		Alm:	AHS < SS	

between the Atlantic and Mediterranean. However, the total abundance showed a different pattern when comparing Atlantic and Mediterranean marinas: it was higher on AHS than in SS in the Mediterranean, but no significant differences were found in the Atlantic (see SNK tests of interaction Lo x Ha in Table 3). When abundances were compared for

each marina, significant differences were detected only in Fuengirola (Fig. 2, see SNK tests of the interaction Ha x Ma (Lo) in Table 3). Shannon-Wiener's Diversity (H') showed a similar pattern to the number of species, with higher values for SS. In this case, significant differences between AHS and SS were detected in all marinas except for Chipiona

and Fuengirola (see SNK tests of the interaction Ha x Ma (Lo)). The twodimensional MDS plot based on species composition and abundances showed segregation of sampling stations mainly by habitats (AHS vs SS) (Fig. 3A). For each habitat, some separation was also detected between Atlantic and Mediterranean marinas. PERMANOVA confirmed significant differences between AHS and SS for all marinas (see pair-wise tests of the interaction Ha x Ma (Lo) in Table 4). Indeed, of the 42 species recorded, only *Hiatella arctica* was present in both habitats; the remaining species were exclusive to AHS (10 species) or to SS (31 species).

Regarding trophic guilds, the bryozoan *B. neritina* was characterized by a filter-feeding mollusc assemblage (Table 1). When AHS and SS were compared along marinas of Southern Iberian Peninsula, AHS was also dominated by filter feeders (Fig. 4). In some marinas, such as Puerto América, Barbate and Almería, the contribution of herbivores was also remarkable. SS was also characterized by an important contribution of filter feeders, but herbivores were replaced by deposit feeders, which were the dominant taxa in Chipiona and Almería. These differences were clearly reflected by PERMANOVA, which showed a similar trend to the PERMANOVA conducted with the mollusc assemblages. Significant differences in the abundance of the different trophic groups were detected between AHS and SS (see pair-wise tests of the interaction Ha x



**Fig. 3.** Non-parametric multidimensional scaling (MDS) showing the relationship among locations (Atlantic vs Mediterranean), habitats (AHS vs SS) and marinas (Chi: Chipiona, Ame: Puerto América, Bar: Barbate, Lin: La Línea, Fue: Fuengirola, Alm: Almería) according to the mollusc assemblages based on species composition and their abundance (A) and based on abundance percentages of each feeding guild (B). Mean values of the three replicates of each pontoon for AHS and SS are included.

#### Table 4

Summary of the four-way PERMANOVA results for the mollusc assemblage (based on taxa composition and abundances). Lo: Location (Atl: Atlantic, Med: Mediterranean); Ma (Lo): Marina nested with location (Chi: Chipiona, Ame: Puerto América, Lin: La Línea, Bar: Barbate, Fue: Fuengirola, Alm: Almería); Po (Ma (Lo): Pontoon nested with marina and location; Ha: Habitat (AHS: artificial hard substrate, SS: soft substrate). df: degrees of freedom; MS mean squares; P (MC): level of significance (Montecarlo): \*\*P < 0.01, \*\*\*P < 0.001, n.s. not significant.

Source of variation	df	MS	Pseudo-F	P (MC)
Lo	1	28,850	2.2513	0.0090**
Ma (Lo)	4	12,682	3.9368	0.0001***
Po(Ma (Lo))	12	3221	1.0938	0.1394 n.s.
На	1	45,853	3.6085	0.0010**
Lo x Ha	1	28,642	2.2540	0.0081**
Ha x Ma (Lo)	4	12,707	3.9447	0.0001***
Ha x Po(Ma (Lo))	12	3221	1.0938	0.1386 n.s
Residual	72	2945		
Total	107			
PAIR-WISE TESTS				
Lo x Ha	AHS:	Atl≠Med		
	SS:	Atl = Med		
	Atl:	AHS≠SS		
	Med:	AHS≠SS		
Ha x Ma (Lo)	Chi:	AHS≠SS		
	Ame:	AHS≠SS		
	Bar:	AHS≠SS		
	Lin:	AHS≠SS		
	Fue:	AHS≠SS		
	Alm:	AHS≠SS		

Ma (Lo) in Table 5). The two-dimensional MDS plot (Fig. 3B) also showed a separation between hard substrates and sediments.

#### 4. Discussion

## 4.1. Mollusc diversity in marinas and sampling methodologies

The present work represents the first comprehensive study of molluscs inhabiting marinas from the Iberian Peninsula. In spite of the high number of marinas surveyed (especially along Southern Iberian Peninsula where both sediments and hard substrates of pontoons were sampled), the total number of molluscs species collected (50) is moderate when compared with vagile mollusc fauna of other ecosystems of Southern Spain. For example, regarding mollusc species associated to seagrasses, González et al. (2007) reported 45 species in three Andalusian seagrass meadows, one of Zostera marina L. (Malaga) and two of Cymodocea nodosa (Ucria) Ascherson (Cádiz and Almería), and Rueda et al. (2009) recorded 162 species associated to Z. marina in the Alboran Sea. Concerning macroalgae, Sánchez-Moyano et al. (2000, 2001) recorded 98 species inhabing Halopteris scoparia (L.) Sauvageau, and 50 species associated to Caulerpa prolifera (Forskal) J.V.Lamouroux both in Algeciras Bay, Cádiz. Dealing with sediment communities, 57 species were found in the sediments of the estuary of Palmones River, Cádiz (Estacio et al., 1999) and a total of 127 species were inhabiting sediments from Algeciras Bay (Estacio 1996). The soft bottoms around mud volcanoes in the Gulf of Cádiz were characterized by the presence of 33 species (Delgado et al., 2013). Urra et al. (2011) reported 243 molluscan species from littoral soft bottoms of the Alboran Sea (Western Mediterranean Sea) indicating that the Southern Iberian coast is highlighted as one of the areas with highest molluscan species richness in Europe. Some of these studies considered a broad number of sedimentary types (e.g. Estacio 1996) that may explain the higher diversity of molluscs.

In relation to marinas, we must point out the lack of information existing on mollusc assemblages in marinas over the world. Although a higher number of studies has been conducted in other harbours and ports, only scarce data are available for marinas. Regarding with artificial hard substrate present in marinas (pontoons, docks, pillars, piers,



Fig. 4. Pie charts comparing mollusc trophic structure (abundance percentages of each feeding guild) between artificial hard substrate (AHS) and soft substrate (SS) at each marina ("Others": carnivores, scavengers and ectoparasites + specialized carnivores).

#### Table 5

Summary of the four-way PERMANOVA results for the mollusc assemblage (based on feeding guilds). Lo: Location (Atl: Atlantic, Med: Mediterranean); Ma (Lo): Marina nested with location (Chi: Chipiona, Ame: Puerto América, Lin: La Línea, Bar: Barbate, Fue: Fuengirola, Alm: Almería); Po(Ma (Lo)): Pontoon nested with marina and location; Ha: Habitat (AHS: artificial hard substrate, SS: soft substrate). df: degrees of freedom; MS: mean square; P (MC): level of significance (Montecarlo): \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, n.s. not significant.

Source of variation	df	MS	Pseudo-F	P (MC)
Lo	1	14,977	2.0056	0.0705 n.s.
Ma (Lo)	4	7468	2.7677	0.0002***
Po(Ma (Lo))	12	2698	1.1972	0.0905 n.s.
На	1	32,675	5.4061	0.0003***
Lo x Ha	1	24,220	4.0071	0.0038**
Ha x Ma (Lo)	4	6044	2.0211	0.0049**
Ha x Po(Ma (Lo))	12	2990	1.3270	0.0194*
Residual	72	2254		
Total	107			
PAIR-WISE TESTS				
Lo x Ha	AHS:	Atl≠Med		
	SS:	Atl = Med		
	Atl:	AHS≠SS		
	Med:	AHS≠SS		
Ha x Ma (Lo)	Chi:	AHS≠SS		
	Ame:	AHS≠SS		
	Bar:	AHS≠SS		
	Lin:	AHS≠SS		
	Fue:	AHS≠SS		
	Alm:	AHS≠SS		

pillings, seawalls) or PVC experimental settling panels placed in marinas little information has been published. Tempesti et al. (2020: supplementary material) collected 35 mollusc species by scraping vertical concrete walls of docks submerged in three marinas of Livorno, Italy. A total of 44 species were registered in dock walls and floating pontoons in two marinas of Ligurian Sea and four marinas of Sardinian Sea, Italia (Ferrario et al., 2017: supplementary material). Kenworthy et al. (2018) listed 7 species from metal pillars of three marinas in Brest, France. Toh et al. (2017) reported 9 species on pontoons of three marinas in Singapore. Five species were recorded using PVC panels in four marinas of Madeira Archipelago (Ramalhosa et al., 2019). The present study reveals 11 species inhabiting pontoons of 6 marinas in Southern Spain (see Table 2). Taking into account that molluscs are not very abundant in hard surfaces of marinas, it seems that quadrat scraping is a more adequate methodology than collecting a particular arborescent substrate. For example, Gavira O'Neill et al. (2015, 2018) studied the seasonal fluctuations during a whole year of the vagile macrofauna

associated to the hydroid *Ectopleura crocea* (Agassiz, 1862) in the marina El Rompido (Huelva) and the bryozoan *Tricellaria inopinata* d'Hondt and Occhipinti Ambrogi, 1985 in the marina Puerto América (Cádiz) and they reported platyhelminthes, annelids and arthropods (mainly amphipods) but no molluscs. During the present study only the bivalve *Sphenia binghami* was found clinging to *B. neritina* in El Rompido and no molluscs were found on *B. neritina* in Puerto América. However, when the surface of Puerto América pontoons was scraped, five species were collected: the bivalves *M. galloprovincialis, M. subpictus, S. binghami*, the gastropod *Runcina* sp. and the polyplacophoran *A. crinita*.

With respect to soft bottom communities of marinas, information about molluscs is usually integrated in studies of larger harbours in which one or some stations correspond to marinas which are located inside these harbours (see e.g. Guerra-García and García-Gómez 2004; Moreira et al., 2005). Soft bottoms of marinas are usually composed of muddy sediments due to the lesser hydrodynamism and high sedimentation rates (Guerra-García and García-Gómez 2005b; Rivero et al., 2013; Gómez et al., 2019). The present study reveals that, comparatively, total mollusc species richness is higher in SS than in AHS (32 vs 11 species in the six marinas sampled in Southern Spain). Indeed, Chatzinikolaou et al. (2018) found that molluscs were the most abundant group in sediments of Mediterranean touristic ports including marinas. Covazzi Harriague et al. (2012) reported 18 species in a single marina of Ligurian Sea, Italy, Guerra-García and García-Gómez (2004) found 14 species in the marina of Ceuta, North Africa, and 15 species were inhabiting the marina of Urla, Turkey (Aydın et al., 2007). Moreira et al. (2010) studied the mollusc fauna of the harbour of Baiona, NW Spain, which is mostly used by recreational and fishing vessels and reported 40 species (see also Moreira 2003). Ng et al. (2019) reported 21 different mollusc families from sediments of three marinas of Singapore. Samples of soft bottom macrofauna in different environments are usually taken with a 0.1 m<sup>2</sup> or 0.05 m<sup>2</sup> van Veen grab (Rumohr 1999; Guerra-García and García-Gómez 2005a). When sediment is rich in sands or gravels this size is necessary since grab must be large and heavy enough to sink properly into the sediments. Marinas are dominated by muddy sediments where grabs can easily penetrate. For this reason, a modified van Veen grab of smaller size (e.g. 215 cm<sup>2</sup>) is recommended. This manageable grab can be effortlessly hand-carried and deployed from the pontoon in the marina. In fact, previous studies successfully used this small grab in marinas (Covazzi Harriage et al., 2012). Either way, other different types of samplers have been also used to collect sediments in marinas (e.g. box corer in Chatzinikolaou et al., 2018; Ekman grab in Ng et al., 2019) and all of them can be adequate to properly represent the macrofaunal communities, which are mainly living in the first centimetres deep in the sediments (Guerra-García and

## García-Gómez, 2008; Guerra-García et al., 2011).

# 4.2. Baseline information as mandatory tool to early detection of exotic molluscs

Harbours and marinas for pleasure craft mooring are considered priority target areas for early detection of NIS; however, in most marine regions of the world, including the Mediterranean Sea, biofouling on recreational boating remains at present an unregulated vector of NIS introduction (Ferrario et al., 2017). The first step before regulation is the accurate knowledge of species inhabiting marinas. Comprehensive lists of taxa are mandatory for subsequent management of potential invaders which could arrive to the marinas.

During the present work, no exotic molluscs were found inhabiting the studied marinas (the 48 species found could be considered native or cryptogenic). To our knowledge, the only introduced molluscs found in marinas of the Iberian Peninsula are, so far, Aplus assimilis (Reeve, 1846) found in the harbours of Sagunto, Sant Carles de la Ràpita, Burriana and Castellón (see Martínez-Ortí et al., 2020), Arcuatula senhousia (Benson, 1842), which has been recorded in One Ocean Port Vell Marina in Barcelona (see Ulman et al., 2017), Magallana gigas (Thunberg, 1793) recorded in El Rompido marina, Huelva (see Guerra-García et al., 2015) and Mitrella psilla (Duclos, 1864) collected in Valencia marina (see Marco Magraner et al., 2020). Brachidontes pharaonis (Fisher, 1870) has been recently reported from ropes of the fishing harbours of Xàbia and Calp (Alicante, Spain) (Martínez-Ortí and Scutia 2021). Marginella glabella (Linnaeus, 1758) is an exotic mollusc from the tropical West Africa established in the southern Mediterranean, which was recorded in the trawler docks of the Malaga harbour (not within the marina) and therefore its introduction seems clearly associated to fishing activities (Luque et al., 2012). The non-indigenous Xenostrobus securis (Lamarck, 1819) has been recorded in the Ria of Pontevedra just a few meters away from the Pontevedra marina (Guerra et al., 2013) and the exotic Haloa japonica (Pilsbry, 1865) has been found in the intertidal area of O Grove, Ria of Arousa, Pontevedra, also close to floating pontoons of Porto do Grove. In fact, A. senhousia, X. securis and H. japonica have been recorded in other Mediterranean marinas of Italian coasts (Ferrario et al., 2017: Tempesti et al., 2020). Recently, the alien nudibranch Anteaeolidiella lurana (Ev. Marcus and Er. Marcus, 1967) was found in a marina of the Old Venetian Harbour of Heraklion, Greece (Bariche et al., 2020). The low number of exotic molluscs in marinas seems to be also applicable to all marine molluscs in Spain; the overall occurrence of non-indigenous molluscs in Spanish waters amounts to hardly 1.5% of the total, despite definitive exposure to vectors such as shipping and aquaculture (Gofas et al., 2017). This finding supports the view that a high number of native species tends to reduce invasion success (Stachowicz et al., 1999 in Gofas et al., 2017).

Although more extensive surveys could probably reveal the presence of additional exotic epifaunal molluscs, their low number along marinas of the Iberian Peninsula seems to be also a general trend for epifaunal polychaetes (e.g. no exotics reported among 32 taxa recorded by Fernández-Romero et al., 2019 in a survey of 42 marinas along the Iberian Peninsula). Indeed, only six sedentary polychate species, of the 23 exotic polychates registered in the Iberian Peninsula, i.e. Branchiomma boholense (Grube, 1878), Branchiomma luctuosum (Grube, 1869), Ficopomatus enigmaticus (Fauvel, 1923), Hydroides dirampha Mörch, 1863, Polydora triglanda Radashevsky and Hsieh, 2000 and Spirorbis marioni Caullery and Mesnil, 1897) have been associated to hull fouling, and only B. luctuosum, F. enigmaticus and P. triglanda have been recorded, so far, in marinas of the Iberian Peninsula (Borrell et al., 2017; López and Richter 2017). This contrasts with the moderate number of exotic crustaceans reported in marinas of the Iberian Peninsula. Only within peracarids, nine exotic species including five isopods, Ianiropsis serricaudis (Gurjanova, 1936), Paracerceis sculpta (Holmes, 1904), Paradella dianae (Menzies, 1962), Paranthura japonica Richardson, 1909 and Sphaeroma walkeri Stebbing, 1905, and four amphipods, Caprella

scaura Templeton (1836), Jassa slatteryi Conlan, 1990, Paracaprella pusilla Mayer, 1890 and Stenothoe georgiana Bynum and Fox, 1977, have been recorded in marinas of the Iberian Peninsula (see Ros et al., 2013b; Martínez-Laíz et al., 2018; Sáenz-Arias et al., 2020).

Regarding molluscs, the faunistic data of the present study provide, therefore, a monitoring baseline for early detection of exotics potentially incoming to the Iberian Peninsula in the near future. Furthermore, the native pool of species found in marinas of Southern Spain include species which could be potentially transported to other areas becoming exotic in other regions (e.g. *M. galloprovincialis*).

## 4.3. Towards an integrate sustainable management in marinas: addressing future mollusc studies

Unfortunately, investment on taxonomic and faunistic studies in marinas is not being sufficiently supported by local governments, policymakers and other authorities, and scientific contributions on this topic are not considered relevant by most of the leading journals. Therefore, the available information of species inhabiting marinas is still very scarce and fragmentary. Moreover, when spatio-temporal benthic biodiversity studies are eventually published, the valuable lists of species are often not provided, nor even in supplementary material (see e.g. Chatzinikolaou et al., 2018). The achievement of these lists requires great dedication, sorting effort and taxonomic expertise, so they should be given more importance. In this sense, the ongoing integrative use of multiple identification techniques (macro-morphological identification, microscopy, and DNA barcoding) maximise taxonomic resolution. Incorporating molecular techniques to characterize biodiversity is especially relevant where cryptogenic and introduced species are common and often unidentified using exclusively morphological tools (Borrell et al., 2017; Kenworthy et al., 2018; Bariche et al., 2020). Complete taxonomical checklists require permanent attention and curating. They must remain up-to-date to allow simple but robust analysis of species richness, giving visibility to the presence of endemic or exotic species, organizing projects dealing with mapping of the distributional data and identifying priority areas for biodiversity conservation and management (Stoch 2000; Gofas et al., 2017). Unlike other phyla such as arthropods, molluscs include many soft-body taxa (e. g. nudibranchs) which are more difficult to be properly fixed and preserved before study. Additional sampling efforts for these groups are, therefore, advisable to accurately characterize the total mollusc biodiversity in marinas to develop further appropriate management strategies. Studies linking biological data to pollution and water quality (Gómez et al. 2017, 2019; Guerra-García et al., 2021b) should be also a priority to understand globally marinas as complex ecosystems.

The present study reflects clear differences in the two main compartments of marinas, AHS and SS. The number of species is higher in SS than in AHS and the species composition is totally different between habitats, with only one species, H. arctica, inhabiting both substrates. This reflects the lack of species flow (e.g. vertical migration) between the two compartments and that both must be treated independently in management strategies of marinas. Herbivorous species were only inhabiting the hard substrate while deposit feeders were restricted to sediments. Carnivorous species were very scarce in the present study. This contrasts with the high number of carnivores in other ecosystems, such as seagrasses (Rueda et al., 2009). The high proportion of carnivores in these beds in comparison with their lack in SS and AHS is probably a reflection of the high biodiversity of invertebrates inhabiting these meadows and, therefore, the higher availability and variety of food sources for carnivores in seagrasses in comparison with habitats of marinas (Templado 1984). Our study also reveals that different marinas do not only have different mollusc composition but also different ecological functioning based on differences in trophic structure. This supports the idea that local scale variability is an important aspect in determining community structure. This pattern of singularity has been also pointed for other artificial marine structures (Sedano et al., 2020),

highlighting the importance of considering local approaches for conservation and management purposes. Indeed, local and small-scale differences within marinas have been suggested as an important factor for management consideration including for some normative certification (e.g. ISO norms, Blue Flag) (Kenworthy et al., 2018).

Species composition was very different between AHS and SS, with only one species in common. Exploration of the different ecosystem compartments has been considered relevant to provide a comprehensive, socio-ecosystem-based approach in the field of environmental management (Boudouresque et al., 2020). Therefore, it is recommended to sample both habitats to get an appropriate representation of global mollusc diversity. Although surveys of AHS and SS are probably a good proxy to the global diversity of molluscs in marinas of the Iberian Peninsula, we focused solely on communities of SS and AHS of the floating plastic pontoons. Although these are the two main habitats, other types of substrates can be found in marinas such as metallic and wooden pillars, concrete walls, rip raps or even natural rocks. Substrate type is known to affect the distribution of organisms, including NIS (e.g. Wasson et al., 2005; Ruiz et al., 2009; Chase et al., 2016). Consequently, future studies should address detailed faunal comparisons among different substrates in marinas through extensive surveys or using experimental panels made of different materials to explore colonization dynamics. For example, floating plastic pontoons (often made of polyethylene) alternate with concrete ones along Mediterranean marinas of the Iberian Peninsula; an exhaustive study of macrofaunal assemblages of both types would help to make decisions in choosing the most environmentally sustainable material for the constructions of new pontoons in the future. This knowledge is necessary to implement better designs of the so-called ecologically-friendly marinas.

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## Availability of data and material

The data and the material is available upon request.

#### 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.

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