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Influence of environmental factors and sessile biota on vagile epibionts: The case of amphipods in marinas across a regional scale

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Abstract

Marinas are highly anthropized environments, with singular ecological characteristics. In the present study the spatial variability of the amphipod assemblage associated to floating pontoons was analyzed. Moreover, the influence of environmental data and sessile biota on the amphipod assemblage structure was also assessed. Six marinas were selected along the Southern Iberian Peninsula, three in the Atlantic and three in the Mediterranean, and three floating pontoons were sampled at each marina. Fourteen amphipod species were identified, most corresponding to detritivores and generalist species. Statistical analyses considering amphipod composition and abundance did not show significant differences between the Atlantic and the Mediterranean, but there were significant differences between marinas. Copper had a significant correlation with amphipod assemblage. The sessile species *Bugula neritina* and *Ellisolandia elongata* also showed significant influence on the spatial patterns of the amphipod assemblages. Therefore, environmental conditions and sessile composition seem to simultaneously affect the spatial variability of amphipod assemblage associated to floating pontoons in marinas. Both factors are key to understanding the singularity of marinas and should be considered in order to prevent the use of a single management program in these environments.

Keywords: Marinas; amphipods; fouling; pollution; copper; exotic species.

Introduction

Nowadays, more than 70% of the human population is concentrated in areas located up to 60 km from the coastline (Evans, 2008). Demographic growth and current human migration patterns predict an increasing anthropic pressure on littoral areas (Airolidi & Beck, 2007). The development of coastal urban centers correlates with the increase in maritime structures related to leisure activities, such as marinas. For example, the number of marinas in Spain increased from 131 in 1976 to 375 in 2015, through heterogenous development along the coast. Specifically, Southern Spain and the Balearic Islands show the highest number of marinas (Federación Española de Asociaciones de Puertos Deportivos y Turísticos, 2016).

Regardless of their design and localization (e.g., geographic area, orientation, material used in its construction, among others) marinas often share similar biota (McKinney & Lockwood, 1999) and environmental features. For example, they show wide oscillations of temperature and salinity, and higher pollution levels than nearby natural environments (Arenas *et al.*, 2006; Bax *et al.*, 2003). Previous environmental studies conducted in marinas have highlighted the relevant role of heavy

metals, especially copper from antifouling paint, as one of the most harmful stressors for marine biota (Amara *et al.*, 2018; Guerra-García *et al.*, 2021). The great amount of submerged artificial surfaces within marinas, such as docks, pilings, pontoons, and buoys, allows for the establishment of many sessile organisms, which could act as basibiont species to other organisms (Minchin, 2012). Sessile communities in these anthropized environments are often dominated by mussels, sea squirts, and arborescent cnidarians and bryozoans (Webb *et al.*, 2000; Orichio *et al.*, 2016). Their presence transforms the bidirectional and laminar plane of the port structure in a complex and tridimensional substrate, increasing the available space and the diversity of microhabitats for small vagile epibionts (Gavira-O'Neill *et al.*, 2016; Cunha *et al.*, 2017). In addition, sessile organisms can also provide food and predator refuge for the vagile community, as well as reproduction and breeding sites (Hay *et al.*, 1987; Bradshaw, 2003; Guerra-García *et al.*, 2015; Cunha *et al.*, 2017, Cunha *et al.*, 2018). Sessile taxa and vagile epibionts together constitute the so-called fouling community (Connell, 2000).

Despite the high abundance and diversity of vagile communities, as well as their key ecological role in such

artificial environments (Sedano *et al.*, 2020a), most of the studies dealing with fouling communities have focused on the sessile species (Chapman *et al.*, 2005; Marchini *et al.*, 2014). This knowledge bias may be due to the relatively small size of most vagile communities (ranging from a few millimeters to two or three centimeters) and their cryptic behavior (e.g., small organisms hiding in the substrate and/or with camouflage ability within the substrate) (Lacerda & Masunari, 2011). Additionally, the taxonomy of some taxa, such as anthurid isopods (Marchini *et al.*, 2014) or caprellid amphipods (Guerra-García *et al.*, 2014a) is very complex, limiting taxonomic resolution (e.g., to supraspecific taxa) or often leading to misidentification. Despite the existence of previous studies encompassing the whole fouling community (e.g., Ferrario *et al.*, 2017; Spagnolo *et al.*, 2019; Tempesti *et al.*, 2020), detailed studies dealing with the vagile community only considered the community associated with a few or even a single sessile species (Conradi *et al.*, 1997; Guerra-García *et al.*, 2015). This approach is useful for spatial and temporal monitoring (e.g., Ros *et al.*, 2013) because it simplifies the characterization of the epifauna. This also minimizes the number of factors involved, for example, the variability between samples is reduced by considering a homogeneous substrate to all regions or study periods (Conradi *et al.*, 1997; Guerra-García *et al.*, 2015). However, selecting only a few or single sessile species is not representative of the dynamics and functioning of the whole fouling community. For example, dominance and composition of the sessile taxa can change both spatially and seasonally (Gavira-O'Neill *et al.*, 2016) and their effects on the associated vagile community are not well known. For this reason, the methodology used in the present study, based on analysis of the whole sessile community, will allow for evaluation of the actual diversity of the fouling community within marinas and the interactions between vagile epibionts and sessile taxa.

Amphipods (Crustacea: Peracarida) are one of the dominant taxa in the vagile community of marinas (Ros, 2015). Their role in benthic ecosystems is highly relevant, being the link between primary producers and many species of fishes (Caine, 1991). Due to their abundance, diversity, ubiquity and sensitivity to environmental changes, amphipods are often used as a model group in benthic ecological studies, acting as bioindicators for a wide variety of natural processes (Guerra-García *et al.*, 2010) and anthropic disturbances, such as chemical pollution (Duffy & Hay, 2000; Guerra-García *et al.*, 2009; Navarro-Barranco *et al.*, 2020). In addition, detritivore amphipods improve the water quality by recycling and redistributing suspended organic matter (Guerra-García & Tierno de Figueroa, 2009). Additionally, amphipods are one of the crustacean taxa with the highest number of exotic species (although the number of exotic species is still underestimated) and they have also been proven to be a useful model group for study of invasion dynamics due to the lack of larval stages, which has relevant implications in their anthropic dispersion. Therefore, amphipods could be a tool to determine biocontamination levels

in anthropic ecosystems such as marinas (Ros, 2015).

The main aims of this study were: i) to describe the spatial variability in amphipod abundance and composition of the fouling community in marinas of the Southern Iberian Peninsula and ii) to determine the influence of environmental parameters (salinity, temperature, pH, turbidity, and chemical elements) and the sessile species of the fouling community on the composition of vagile amphipods. Specifically, the present study tested the following hypothesis: i) amphipod communities are unique in each marina and ii) both the aforementioned environmental parameters and the sessile species of the fouling constitute key factors determining amphipod distribution and structure.

Materials and Methods

Study sites and sampling procedure

The study was carried out between June and July 2017 in six marinas: Chipiona (36.74611° N, 6.42936° W), Puerto América (36.54294° N, 6.28250° W) and Barbate (36.18411° N, 5.93269° W) in the Atlantic Ocean and La Línea (Puerto Chico) (36.16075° N, 5.35558° W), Fuengirola (36.54162° N, 4.61465° W) and Almería (36.83377° N, 2.46558° W) in the Mediterranean Sea. All the selected marinas are recreational marinas with leisure activities, and all have wood floating pontoons with lateral floating structures made of plastic, where the samples were collected. Details for these marinas (map, surface, number of births and population density) are included in Guerra-García *et al.* (2021). Three different pontoons were haphazardly selected in each marina. Three random grids (15x15cm) were put on the discontinuous lateral section of the submerged surface of each pontoon. The grids were placed just below water level and the fouling community was scraped with a spatula directly from the pontoon, with a collector placed below to avoid the loss of the sessile species and their associated epibiota before preservation in 96° ethanol. Three independent measures of physical-chemical parameters (salinity, temperature, pH, and turbidity) were also taken *in situ* at each pontoon and mean value and standard deviation were calculated for each parameter. Temperature (°C) and salinity (psu) were measured using a conductivity meter WTW LF 323-A/Set, pH with a pH meter WTW pH 300i/Set, and turbidity (ntu) with a turbidimeter WTW TURB 355 IR. Additionally, three 150 ml seawater samples were taken at each marina to measure total organic carbon (TOC) and other elements such as Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, P, Pb, S, Sr and Zn.

Laboratory procedures

Each fouling replicate was washed through a 500 µm mesh for the collection of macrofaunal organisms. Vagile species were separated from sessile species and sorted by phyla. Amphipods were identified to the species

level and their abundance was expressed as the number of individuals per 1000 ml of substrate, due to the different structure of the secondary substrate species (Pereira *et al.*, 2006). Species names follow the World Register of Marine Species (WoRMS, 2021). Each species of amphipod was also associated to a frequency of occurrence (FA), defined as the percentage of collected replicates in which the species was present. The abundance of the species in relation to the total amphipod abundance (TAA) was also calculated. Sessile species were identified to the lowest possible taxonomical level and their volume was estimated as the difference between the initial and final volumes when placed into a narrow-graduated cylinder with a fixed volume of water.

Seawater samples were filtered through nylon filters (pore size = 0.45 μm) and acidified with 2% HNO_3 (30%). Total concentrations of major, minor and trace elements were determined by Inductively Coupled Plasma-Optical Emission Spectrometer (Varian ICP-OES ICP 720-ES). TOC measuring was performed with a TOC-auto analyzer (Shimadzu TOC-VCSH) after filtration by Whatman paper (pore diameter = 11 μm) and samples were injected into the analyzer via an auto sampler (Shimadzu ASI-V) (according to Ros *et al.*, 2015). The accuracy of the analytical methods was assessed using reference water samples (TR- 434 Trace of metals in drinking water from INTER 2000 Program and Trace Elements in Estuarine Water CRM 505 No. 048). Values of environmental data are provided in Guerra-García *et al.* (2021).

Statistical analyses

The statistical design for the analysis of spatial variation of the amphipod community included three factors: 'Region' (Re), a fixed factor with two levels (Atlantic vs Mediterranean); 'Marina' [Ma(Re)], a random factor with three levels nested in Re (three marinas located within each region); 'Pontoon' [Po(Ma(Re))], a random factor with three levels, corresponding to the three pontoons randomly selected within each marina ($n = 3$). For each replicate, the number of amphipod species (S), abundance (ind/1000 ml) and Shannon-Wiener diversity values (H' , $\log e$; Shannon & Weaver, 1999) were calculated. A three-way ANOVA was carried out to test for differences among factors for these parameters. Before the ANOVA, a Cochran's test was conducted to check the homogeneity of variances (Winer *et al.*, 1991). When heterogeneity of variance was detected, data were transformed with $\log(x + 1)$. Normality was not checked because ANOVA is a robust enough test for even non-parametric data in balanced experiments (Underwood *et al.*, 2002). When ANOVA detected significant differences for a given factor or interaction of factors, the Student-Newman-Keuls test (SNK) was used to identify the source of said differences. Univariate analyses were conducted with GMAV-5 (Underwood *et al.*, 2002; Schmider *et al.*, 2010).

Differences in the structure of the amphipod communities were explored by permutational multivariate analysis of variance (PERMANOVA) using the same hi-

erarchical design as described above (Clarke & Gorley, 2001). PERMANOVA analyses were conducted using the Bray-Curtis similarity matrix on the square root-transformed abundance data, in order to reduce the relative importance of dominant species and increase the contribution of less abundant species. Furthermore, an additional PERMANOVA was conducted using presence/absence data to study those differences caused only by the species composition. Significant factors were examined through pair-wise tests. Additionally, a permutational analysis of multivariate dispersion (PERMDISP) was carried out to test for differences in the variation of the amphipod community among pairs of levels of the considered factors. A non-parametric multidimensional scaling (MDS) (Clarke & Warwick, 1994) using square-root transformed data was also carried out to show the relationship among regions and marinas. MDS was supplemented with a similarity profile analysis (SIMPROF) (Clarke *et al.*, 2008) to identify significant groups of replicates. The DistLM (Distance-based Linear Models) routine was used to assess the relative contribution of abiotic parameters to explain the variability observed in the amphipod assemblage (Anderson *et al.*, 2008). The analysis was performed based on a fourth-root abundance matrix and the normalized environmental data (previously transformed with $\log(x + 1)$). Data for each marina ($n = 6$) was calculated using the mean value of the three pontoons of each marina. Results of the BEST selection procedure, which select the best possible combination of predictor variables based on the AIC (Akaike's Information Criteria) criterion, was included (Burnham & Anderson, 2004; Mulik *et al.*, 2020). Additional analyses were also conducted using forward and step-wise selection procedures based on BIC (Bayesian Information Criterion) and adjusted R² to explore the consistency of the obtained results. We then proceeded in a similar fashion to assess the contribution of sessile taxa of the fouling community to the variability of the amphipod assemblage. Prior to the analyses, multicollinearity between abiotic factors (and between substrates of the sessile community) was tested using Draftsman plots based on Spearman correlation. Variables showing significant correlation ($P < 0.05$) were not included in the analyses. The resulting models ('amphipods vs environmental data' and 'amphipods vs sessile community') were represented in a multi-dimensional space with redundancy analysis (RDA) plots. Multivariate analyses were conducted with PRIMER v6 + PERMANOVA (Clarke & Gorley, 2001).

Finally, the biocontamination level for amphipod assemblage at each pontoon was determined using the Biocontamination Index (SBCI) according to Arbačiauskas *et al.* (2008). This index is based on the Abundance Contamination Index (ACI) and Richness Contamination Index (RCI). ACI was calculated as the number of exotic species divided by the number of total species. RCI was calculated as the abundance of exotic species divided by the total abundance. Both indices generated a matrix with five levels of biocontamination: 0 (no biocontamination or "high" ecological status), 1 (low biocontamination or "good" ecological status), 2 (moderate biocontamination

or “moderate” ecological status), 3 (high biocontamination or “poor” ecological status) and 4 (severe biocontamination or “bad” ecological status) (Arbačiauskas *et al.*, 2008).

Results

Vagile community

A total of 14806 vagile individuals were found in association with the sessile taxa. Arthropoda was the dominant phylum (89.16% of the total abundance), followed by Annelida (5.94%), Mollusca (4.24%) and Echinodermata (0.28%). A few specimens (0.38%) were not determined to the phylum level due to poor conservation. Within Arthropoda, peracarids (Crustacea: Malacostraca) were the most abundant taxa. Three orders of peracarids included more than 99.5% of all crustacean individuals: Amphipoda (81.64%), Isopoda (11.78%) and Tanaidacea (6.5%). Remaining crustaceans corresponded to Decapoda (0.08%).

Regarding Amphipoda, a total of 10417 individuals be-

longing to 14 species were identified during the present study (Fig. 1). *Elasmopus rapax* Costa, 1853 and *Laticorophium baconi* Shoemaker, 1934 were the dominant species, representing 50% - 90% of the total abundance in all marinas except for La Línea, where *Caprella scaura* Templeton, 1836 showed the highest abundance. Four exotic species were detected: *C. scaura*, *L. baconi*, *Jassa slatteryi* Conlan 1990, and *Stenothoe georgiana* Bynum & Fox, 1977 (Saenz-Arias *et al.*, 2020 and references therein). All marinas had at least two exotic species; La Línea and Almería showed the highest numbers (four each). *L. baconi* had 96% FA and accounted for 40.57% of TAA, followed by *C. scaura* (46% FA; 8.37% TAA), *J. slatteryi* (22% FA; 1.43% TAA) and *S. georgiana* (24% FA; 0.91%). The biocontamination index showed high values in all marinas; the highest value was found in Puerto América (SBCI = 4; severe biocontamination) (Table 1).

ANOVA results showed similar trends for number of species (S), abundance of amphipods (N) and Shannon-Wiener diversity (H') values. Factors ‘Region’ and ‘Pontoon’ ($F = 1.27$; $P > 0.05$) did not have significant influence on such parameters while there were differenc-

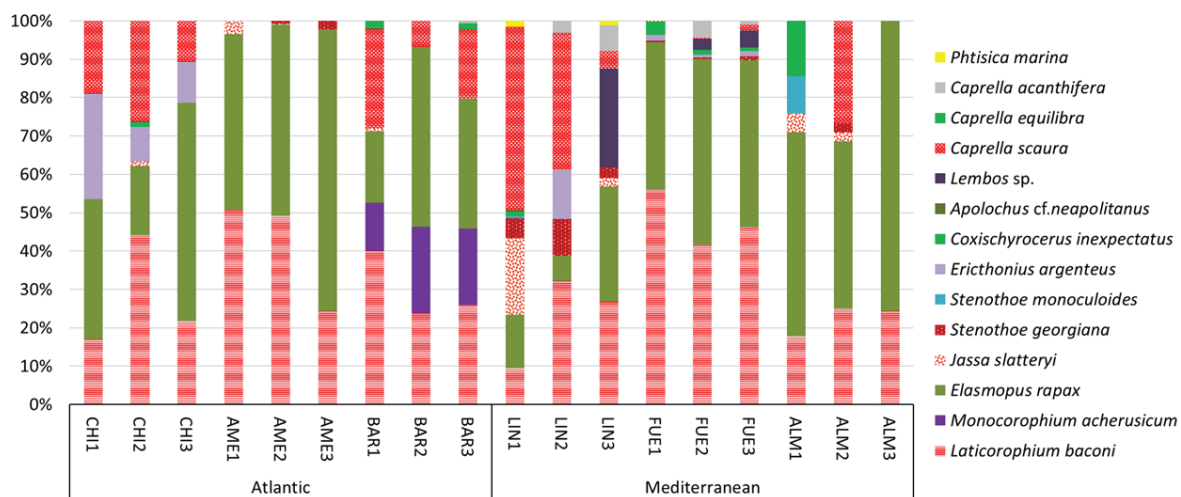


Fig. 1: Composition (percentage of total abundance) of amphipod assemblages occurred on pontoons of each marina (CHI = Chipiona, AME = Puerto. América, BAR = Barbate, LIN= La Línea, FUE = Fuengirola, ALM = Almería. Numbers represents the three pontoons). Exotic species are represented by red textures.

Table 1. Biocontamination index (SBCI) for pontoons and marinas from the Atlantic (A) and Mediterranean (B) (Orange = high biocontamination; Red = severe biocontamination) based on amphipod composition. Values corresponding to Blue (no biocontamination), Green (Low biocontamination) and Yellow (Moderate biocontamination) status were not found in the sampled marinas.

A		Chipiona			Pto. América			Barbate		
SBCI (marina)		3.33±0.58			4.00±0.00			3.33±0.58		
SBCI (pontoon)		P1	P2	P3	P1	P2	P3	P1	P2	P3
		3	4	3	4	4	4	4	3	3
B		La Línea			Fuengirola			Almería		
SBCI (marina)		3.67±0.58			3.67±0.58			3.33±0.58		
SBCI (pontoon)		P1	P2	P3	P1	P2	P3	P1	P2	P3
		4	4	3	4	3	4	3	4	3

es for the factor ‘Marina’ (Fig. 2; Table 2). According to SNK results ($P < 0.05$), all marinas showed different values of amphipod abundance, except for Puerto América and Barbate. Concerning species richness and diversity values, Almería and Puerto América were the only marinas with significant differences: Almería in terms of number of species and Pto. América in diversity.

PERMANOVA also showed significant differences among marinas but not for factors Region or Pontoon (Table 3). Pair-wise tests revealed that all marinas were significantly different from each other. PERMDISP results showed the existence of significant differences in homogeneity among marinas ($F = 6.04$; $P < 0.001$). MDS showed a tendency for some replicates belonging to the same marina to group together and general overlap between the Atlantic and Mediterranean replicates (Fig. 3). SIMPROF test showed two significant groups ($P < 0.05$): one formed by almost all the replicates from Almería and another encompassing the rest of the replicates.

Sessile community

The composition of the sessile assemblage was heterogeneous among different marinas (Fig. 4). Three out of six marinas clearly showed a dominant species: *Amathia verticillata* (delle Chiaje, 1822) in Chipiona (85.26%), *Bugula neritina* (Linnaeus, 1758) in La Línea (66.36%) and *Ellisolandia elongata* (J. Ellis & Solander) K. R. Hind & Saunders, 2013 in Almería (100%). The remaining sessile species were *Mytilus galloprovincialis* Lamarck, 1819; *Ecteinascidia turbinata* Herdman, 1880; *Tricellaria inopinata* d’Hondt & Occhipinti Ambrogi, 1985; Other ascidians and Filiform algae.

The results of the Draftman plots showed significant correlation between *T. inopinata* with *A. verticillata*, and “Other ascidians” with *B. neritina*, so they were not included in the DistLM. Therefore, the model was run with 5 variables: *B. neritina*, *E. elongata*, *E. turbinata*, *A. verticillata*, *Dictyota* sp., *M. galloprovincialis* and Filiform

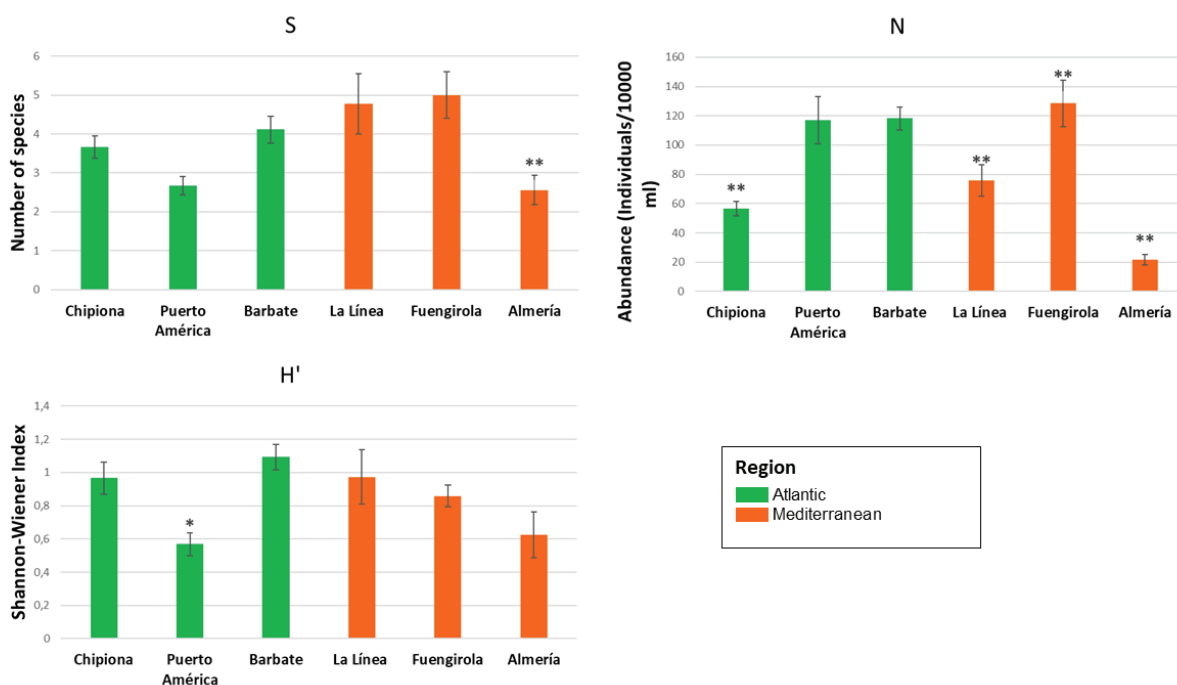


Fig. 2: Number of species (S), abundance (N) and Shannon-Wiener diversity values (H') of the amphipod community (Mean \pm standard error). * $P < 0.05$; ** $P < 0.01$.

Table 2. Results of the ANOVA analyses conducted for several univariate parameters determined after the amphipod assemblage. Re = Region (Atl: Atlantic, Med: Mediterranean); Ma(Re) = Marina nested in region (Chi: Chipiona, Ame: Puerto América, Lin: La Línea, Bar: Barbate, Fue: Fuengirola, Alm: Almería); Po (Ma(Re)) = Pontoon nested with marina and region. Significant values are in bold.

Source of variation	df	Number of species			df	Abundance			df	Shannon-Wiener Index		
		MS	F	P		MS	F	P		MS	F	P
Re	1	6466.1873	0.50	0.5182	1	5.3519	0.35	0.5854	1	43.2934	0.09	0.7782
Ma (Re)	4	18418.4058	4.37	0.0207	4	10.6852	14.87	0.0001	4	477.7775	3.44	0.0429
Po (Ma (Re))	12	1238.8050	1.24	0.2781	12	2.4444	1.22	0.3074	12	138.77283	1.47	0.1823
Residual	36	1015.1641			36	1.9259			36	94.6251		
Total	53				53				53			

Table 3. Results of the PERMANOVA analyses conducted for amphipod assemblage; data transformed by square root (A) and presence/absence (B). Re = Region (Atl: Atlantic, Med: Mediterranean); Ma(Re) = Marina nested in region (Chi: Chipiona, Ame: Puerto América, Lin: La Línea, Bar: Barbate, Fue: Fuengirola, Alm: Almería); Po (Ma(Re)) = Pontoon nested with marina and region. Significant values are in bold.

Source of variation	df	MS	Pseudo- <i>F</i>	<i>P</i>
(A)				
Re	1	5647.5	0.53258	0.7692
Ma (Re)	4	10604	9.8899	0.0001
Po (Ma (Re))	12	1072.2	1.3284	0.0585
Residual	36	807.15		
Total	53			
Pair wise Ma (Re)				
	Atl: CHI ≠AME≠BAR			
	Med: LIN≠FUE≠ALM			
(B)				
Re	1	2184	0.43443	0.6741
Ma (Re)	4	5027.4	7.6238	0.0001
Po (Ma (Re))	12	659.44	1.4979	0.0983
Residual	36	440.23		
Total	53			
Pair wise Ma (Re)				
	Atl: CHI ≠AME≠BAR			
	Med: (LIN=ALM) ≠FUE			

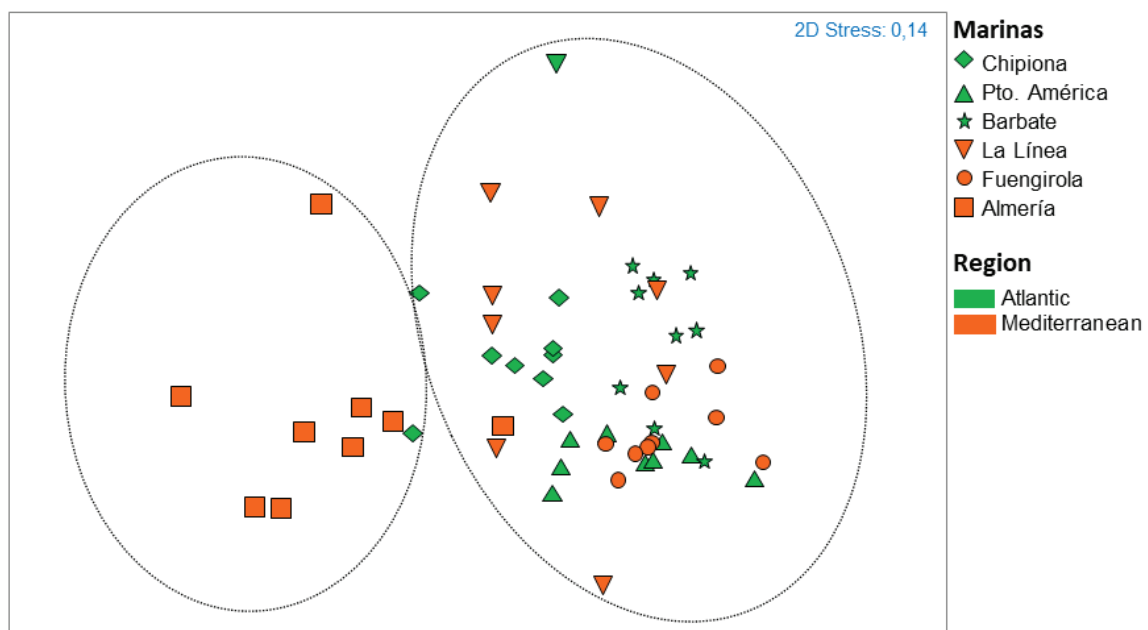


Fig. 3: Two dimensional MDS plot based on Bray–Curtis similarity matrix on the square root-transformed amphipod abundance data. Dashed lines indicate SIMPROF results ($P < 0.05$).

algae. The only sessile taxa which significantly correlated with amphipod assemblages were *E. elongata* ($SS = 1579.7$, $Pseudo-F = 2.1084$, $P < 0.01$) and *Bugula neritina* ($SS = 1573.9$, $Pseudo-F = 2.0966$, $P < 0.05$). The best model ($AIC = 33.292$, $R^2 = 0.93639$, $RSS = 291.11$) was obtained for the combination of four sessile species (*B. neritina*, *E. elongata*, *A. verticillata* and *Dictyota* sp.) (Fig. 5). The first two axes explained 65.8% of the total variation.

Environmental variables

The following excluded variables showed correlation with the variables indicated in brackets: Co (P), Mn (Al, Fe, Zn and Turbidity), Fe (Al, Mn, S Zn, Salinity, pH and Turbidity), As (Turbidity), TOC (Ni, Salinity, Temperature, pH and Turbidity), Sr (Cr, S, Salinity, pH and Turbidity) and pH (Fe, S, Sr, TOC, Salinity, Temperature and Turbidity). These were not included in the DistLM so the model was run with 11 variables (Al, Cd, Pb, Turbidity, Temperature, Salinity, S, P, Ni, Cu, Zn and Cr). The only

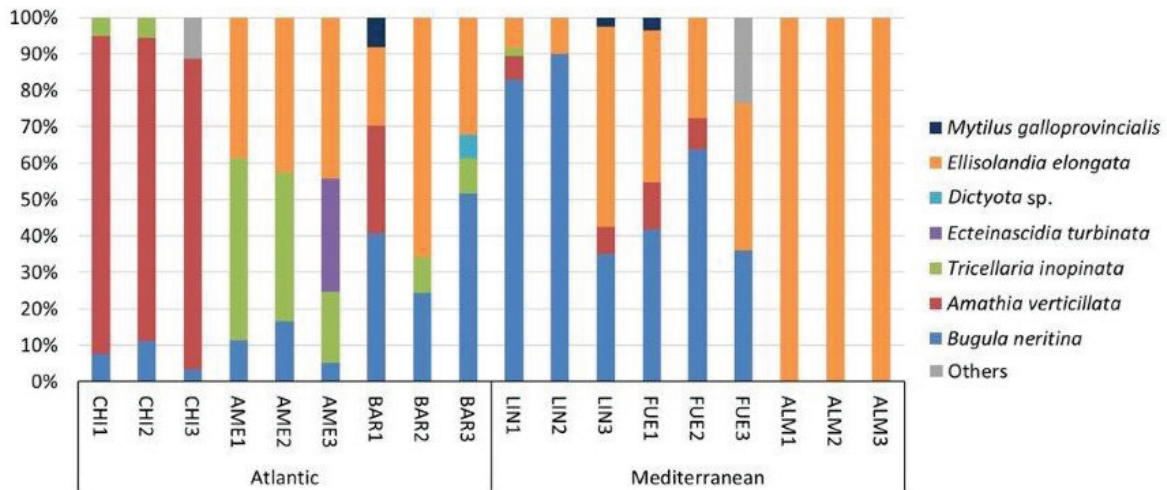


Fig. 4: Composition (percentage of total volumen) of sessile species present in the pontoons of each marina (CHI = Chipiona, AME = Puerto. América, BAR = Barbate, LIN= La Línea, FUE = Fuengirola, ALM = Almería. Numbers represents the three pontoons). The group “Others” includes “Other ascidians” and “Filiform algae”.

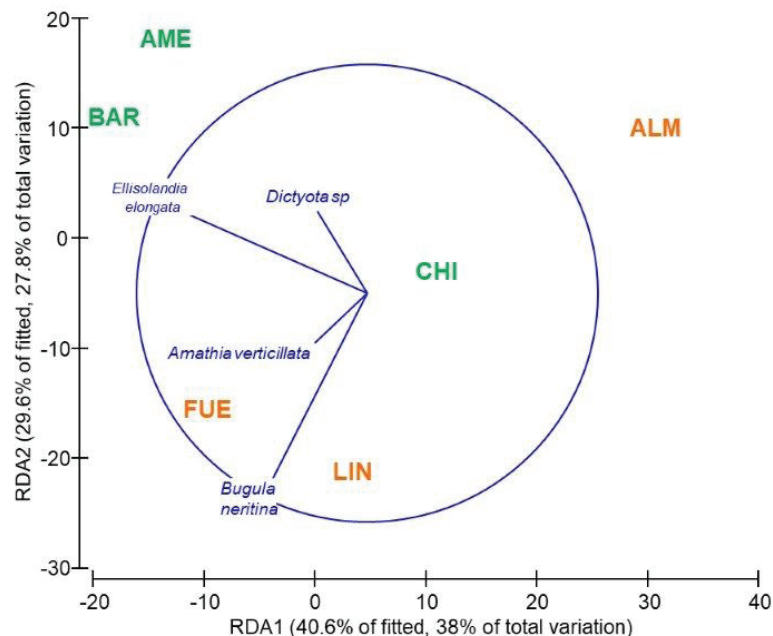


Fig. 5: RDA graphic showing the similarity between the amphipod community present in each marina and its relationship with the sessile community (CHI = Chipiona, AME = Puerto. América, BAR = Barbate, LIN= La Línea, FUE = Fuengirola, ALM = Almería; Green = Atlantic; Orange = Mediterranean).

environmental variable that significantly correlated with amphipod assemblages was Cu (SS = 1570.4, *Pseudo-F* = 2.0895, $P < 0.05$). The best model (AIC = 30.974, $R^2 = 0.05677$, RSS = 197.84) was obtained for the combination of only 4 variables: Cu, Al, Zn and S (Fig. 6). The first two axes explained 65.1% of the total variation.

Discussion

Characterization of the amphipod assemblage

The vagile community associated to pontoons was clearly dominated by amphipods. However, the number of amphipod species was lower in marinas than on the

natural hard substrates that had previously been investigated within the study area (Guerra-García *et al.*, 2011; Izquierdo *et al.*, 2011; Navarro-Barranco *et al.*, 2018). Most of the amphipods recorded were generalist species with a wide distribution in temperate waters (Conradi *et al.*, 1997). *E. rapax*, the dominant species throughout the survey, has often been reported in temperate and subtropical seas around the world (Hughes & Lowry, 2010). The native range of *E. rapax* is unknown and it is usually considered cryptogenic (a species with an uncertain native distributional range) in some locations. However, it is also considered to be invasive in other locations, such as Australia (Hughes & Lowry, 2010). The difficulty to assess its biogeographic status lies in the existence of a complex of species for *E. rapax* (Senna & Souza-Filho, 2011 and

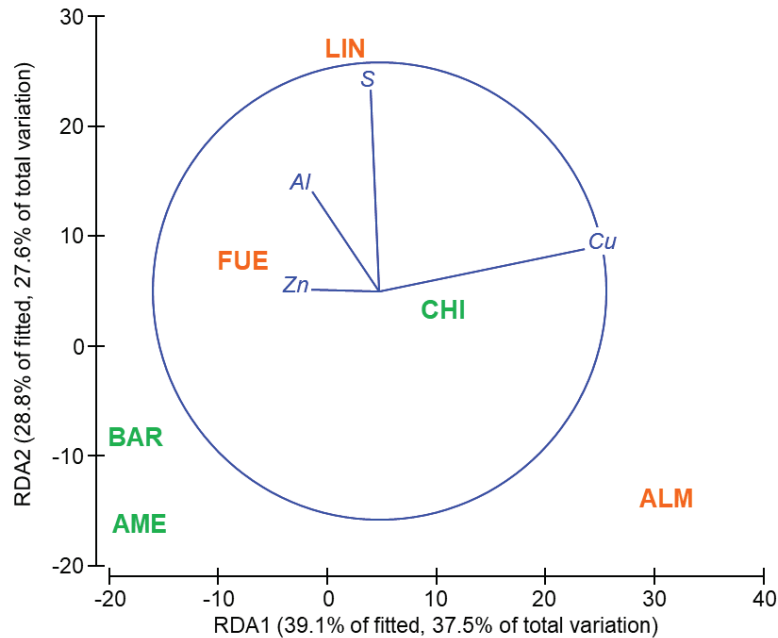


Fig. 6: RDA graphic showing the similarity between the amphipod community present in each marina and its relationship with the physical-chemical parameters (CHI = Chipiona, AME = Puerto. América, BAR = Barbate, LIN= La Línea, FUE = Fuengirola, ALM = Almería; Green = Atlantic; Orange = Mediterranean)..

references therein). In the present study we have considered *E. rapax* as a cryptogenic species and have not included it in the calculation of the biocontamination index. This species has been frequently associated with ships, marinas and other artificial substrates along the Southern Iberian Peninsula and the Mediterranean Sea, suggesting that its distribution range may have been expanded through recreational boating traffic (Martínez-Laiz *et al.*, 2019; Sedano *et al.*, 2020b). The other dominant amphipod, *L. baconi*, was first described in California and is currently considered exotic in the Asian Pacific, Atlantic Oceans and Australia (Hirayama, 1986; Bousfield & Hoover, 1997; Lecroy, 2004; Valério-Berardo & De Souza, 2009; Ah Yong & Wilkens, 2011). Recently, Gouillieux & Sauriau (2019) found individuals of *L. baconi* associated to buoy-fouling communities in a marina in Tarragona (Spain), which was the first record of this species in the Mediterranean. The present study represents the first record of *L. baconi* on the Atlantic coasts of the Iberian Peninsula. However, *L. baconi* has been easily confused with *Apocorophium acutum* (Chevreux, 1908) (Gouillieux & Sauriau, 2019), so *L. baconi* could already be present in several other places in the Mediterranean but have been misidentified as *A. acutum*.

Most of the species reported in the present study are mainly detritivores, except for the carnivorous *Apolochus neapolitanus* (Della Valle, 1893) (Guerra-García & Tierno de Figueroa, 2009; Guerra-García *et al.*, 2014b). The trophic ecology of the exotic species *L. baconi*, *J. slatteryi* and *S. georgiana* has not been studied yet. In the study conducted by Guerra-García *et al.* (2014b) all the analyzed members of the Corophiid family were detritivores, including *Monocorophium acherusicum* (Costa, 1853) (present in the conducted study) and *A. acutum* (a species that is morphologically very similar to *L. baconi*).

In addition, several species found in the present study, such as the genus *Jassa* or *L. baconi*, use the detritus and secretions to build tubes of organic matter where they live (Armsby & Tisch, 2006; Gouillieux & Sauriau, 2019). For these reasons, the high levels of suspended organic matter in marinas may support the occurrence of detritivores organisms.

Four exotic species were found in the whole study and the biocontamination index values (calculated for amphipods) were “high” or “severe” for all sampled pontoons (Table 1). Exotic species represent a frequent element among the amphipod assemblage of marinas (e.g., Gavrira-O’Neill *et al.*, 2016; Ros *et al.*, 2013; Ros *et al.*, 2015). The transport of organisms via hull fouling from recreational boats is one of the main vectors for introduction of exotic species in marinas (Bax *et al.*, 2003) and their establishment in these habitats is favored by their tolerance to perturbations (Kenworthy *et al.*, 2018). In fact, there is a positive relation between the degree of maritime traffic or Index of Recreational Port Capability (RPCI) (measured as the number of available moorings by marina in a given area of coastline; see Occhipinti-Ambrogi, 2002) with the abundance of exotic species (Minchin *et al.*, 2006). Moreover, the low hydrodynamism and the availability of submerged surfaces increase the retention and the propagule pressure of planktonic larvae from sessile fouling species in the marina (Floerl & Inglis, 2003). Additionally, there are eco-ethological factors promoting the successful establishment of some of the exotic species recorded here. For example, the success of *C. scaura* seems to be related to maternal care (Lim & Alexander 1986), aggressive behaviour (Schulz & Alexander 2001) and a flexible diet according to the habitat (Ros *et al.*, 2014).

Factors determining the amphipod assemblage

The low diversity of the vagile assemblage on marinas has been related to great anthropic pollution (e.g., heavy metals and nutrient enrichment) (Fernández-Romero *et al.*, 2019). Although some metals are needed for the metabolism of marine organisms (e.g., as constituents of enzymes) (Jakimska *et al.*, 2011), high concentrations of metals reduce the abundance of native specialist species while favoring generalist and opportunist ones (Johnston & Roberts, 2009). For example, Conradi *et al.* (2000) showed that in a highly anthropized bay the amphipod community was more homogeneous in the inner areas (where the hydrodynamism and the water renewal are lower) with a high degree of pollution. Following this pattern, a lower degree of spatial heterogeneity in the assemblage of amphipods among marinas could be expected, and, for example, high levels of pollution would lead to biotic homogenization of these environments. However, the results of ANOVA and PERMANOVA revealed that 'Marina' was the only significant factor. In addition, pair-wise tests showed that all the marinas were different from each other. These results agree with Martínez-Laiz *et al.* (2019), which also highlight the marked spatial heterogeneity in the composition of peracarid species across Mediterranean marinas. A similar pattern has also been highlighted for sessile communities (Ferrario *et al.*, 2017; Tempesti *et al.*, 2020). Although metal pollution tends to simplify the communities, these results point out the existence of intrinsic factors within each marina that affect abundance and composition of the amphipod community. Even though PERMANOVA results for balanced designs are quite robust to heterogeneity of multivariate dispersions among groups (Anderson & Walsh, 2013), PERMDISP on the factor 'Marina' was highly significant, revealing marked differences in the internal variability among different marinas. These differences in the internal variability were also highlighted in MDS, which showed different degrees of grouping of samples from different marinas. Furthermore, groups of samples belonging to different marinas showed a strong overlap (confirmed by SIMPROF). These results support the local-scale variability within marinas reported by other authors in fouling communities at inner and outer zones of each marina (Kenworthy *et al.*, 2018).

Environmental data

The DistLM considering physical-chemical parameters showed that Al, S, Zn and Cu explained the variations in the amphipod assemblage, but only Cu was significantly correlated. The 2008 prohibition on Tributyltin (TBT) in antifouling paints due to its deleterious effect on marine organisms (e.g., endocrine disorders in gastropods or malformations in oysters; IMO, 2001) increased the use of copper as a biocide base in such paints. Nowadays, the existence of boats treated with such products is considered to be the main source of copper in marinas (Biggs & D'Anna, 2012). However, the copper-based

treatment does not only affect treated surfaces because the reagents are absorbed by suspended particles, and the metal remains available to the benthic community (Weis & Weis, 1994). For this reason, the highest concentrations of copper are found in marinas with high activity (based on the number of moorings, traffic density, etc.) and low hydrodynamics (Brooks & Waldo, 2009). Moreover, copper pollution is one of the reasons for the success of exotic species in marinas due to higher tolerance to high levels of this metal in comparison to native species (Crooks *et al.*, 2010). Zinc, one of the important elements according to our study, is also a component of antifouling paints (Moreira *et al.*, 2017). Similar to copper, zinc particles can come off the boat hull and tend to bioaccumulate in the tissues of filter feeders (Turner *et al.*, 2009). Furthermore, sulfur is a non-metal element present in hydrocarbons such as ship fuels (Tao *et al.*, 2013). Although fuels used in recreational boats have less sulfur content than the ones used in commercial ships (e.g., gasoline or diesel; Junta de Andalucía, 2015), they still produce the emission and solubilization of these pollutants, increasing concentrations in marinas' water. Concerning aluminum, it mostly comes from pipes and other marina structures, however, aluminum is a very conservative element, and its concentration is not dependent on pollution. To this point, the values of aluminum content are used for normalization of data related to heavy metals (Ho *et al.*, 2012).

In contrast to previous studies focused on fouling communities in marinas, our results did not reflect a significant effect of salinity and temperature in the amphipod community (e.g., Ros *et al.*, 2015; Chatzinikolaou *et al.* 2018). This could be explained by the relatively small spatial scale of the study (see e.g., Ros *et al.*, 2015). Although our study sampled marinas in the Atlantic and Mediterranean, the variability of these parameters is lower than in the previous studies cited above. Thus, our results suggest that local factors such as the concentration of heavy metals would affect vagile communities on a regional scale, and others, like salinity and temperature, would have more effect on a larger geographical scale.

Sessile community

The main component of the sessile community corresponded to filter feeders taxa (71.4% of the sessile species and the 48.9% in terms of relative abundance), such as *B. neritina* or *T. inopinata* that are exotic species frequently found in marinas and other habitats with low environmental quality (García-Gómez, 2015; Gavira-O'Neill *et al.*, 2016). The shortage of algae observed in marinas has been associated with the reduced light availability caused by high turbidity conditions (Airoldi & Cinelli, 1997). In fact, the most abundant alga was *E. elongata*, which can tolerate high levels of organic pollution and sedimentation (Arévalo *et al.*, 2007).

The DistLM for sessile species showed that a high percentage of the variability of the amphipod community could be explained by *B. neritina*, *E. elongata*, *A. verti-*

cillata and *Dictyota* sp. The singularity of the amphipod community in the marina of Almería (see Fig.3) could be due to overwhelming dominance of *E. elongata* there.

Differences in the sessile community often produce changes in the abundance of vagile species rather than in overall species composition (Gestoso *et al.*, 2010). Patterns in total and relative amphipod abundances among different sessile species could be explained by features of the sessile taxa, including degree of compactness, spatial complexity or chemical and structural defenses (Ólafsson, 2016). Although there is a correlation between the sessile and the vagile assemblage, a high proportion of marine vagile species are associated with multiple sessile species which are functionally equivalent (Hay & Fenical, 1988; Saarinen *et al.*, 2018). The two dominant amphipod species in the present study, *E. rapax* and *L. baconi*, are cosmopolitan species that have been found associated with different sessile species and artificial structures (Hughes & Lowry, 2010; Gouillieux & Sauriau, 2019). This generalist behavior is also shown for most of the species recorded in the present study. For example, Guerra-García (2001) showed that many species of caprellid amphipods are not exclusive to certain substrates they inhabit. Although Ros *et al.* (2013) highlighted the preference of *C. scaura* for *B. neritina*, the authors recorded this amphipod in the four dominant sessile species of the marina too.

Conclusions

The vagile assemblage of fouling communities in marinas was clearly dominated by amphipods. Among the different spatial scales considered (e.g., biogeographical region, marinas, pontoons within each marina), marina was the only significant factor. Thus, even marinas located in the same region and separated by few kilometers have unique faunal communities. This fact is paramount when developing management strategies for these habitats. Amphipod assemblages in marinas' fouling are conditioned by several environmental parameters and substrate composition. Copper, which is abundant in antifouling paints, was the environmental parameter that most correlated with amphipod assemblage. Contrary to our predictions, salinity and temperature did not significantly affect amphipod assemblage, possibly because their effect is only noticeable when considering wider scales. In addition, a temporal perspective is needed to establish the real effects of the sessile species and the heavy metals in the amphipod assemblage. The values of the biocontamination index were quite high, suggesting that marinas are reservoirs of exotic species and therefore contribute to their spread to other areas through leisure ships or recreational boats.

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