



## Marinas: An overlooked habitat for exploring the relation among polychaete assemblages and environmental factors

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### ABSTRACT

The increasing number of anthropogenic areas in the marine environment results in significant impact to adjacent ecosystems. In fact, the presence of marinas modifies the original environmental conditions and ends up disturbing the faunal community. However, despite the essential role displayed by the macrofauna on marinas' fouling biota, certain taxa such as polychaetes have been poorly studied. The present study provides the first spatial characterization of the epibiont polychaete fauna associated with the bryozoan *Bugula neritina* in marinas along the Iberian Peninsula and the north of Morocco. A total of 32 polychaete species were identified, with Syllidae being the most diverse family. Furthermore, the environmental factors involved in the occurrence and abundance of the dominant species *Salvatoria clavata* were also analyzed by Generalized Linear Models; results showed that the highest predicted values of *S. clavata* abundance appeared at marinas with high levels of nutrient enrichment and of heavy metals concentration.

### 1. Introduction

Nowadays coastal areas worldwide are under great anthropogenic pressure. Recent estimations indicate that > 70% of the human population currently lives in areas located < 60 km from the coastline (Small and Nicholls, 2003; Evans, 2008). Additionally, urbanization of coastal areas is expected to increase in the near future as a result of ongoing demographic expansion and the tendency to migrate to areas by the sea (Airoldi and Beck, 2007; Vaselli et al., 2008). Consequently, the demand for urban infrastructures on the coastline has also increased considerably, which has resulted, in turn, in a significant impact on adjacent marine ecosystems (Bulleri and Chapman, 2010).

In fact, marinas have experienced one of the greatest rates of development in recent years because of increasing tourist activity in coastal areas around the world (Airoldi and Beck, 2007; Callier et al., 2009). Marina construction involves building a high amount of artificial structures in the marine environment, from dykes or breakwaters that provide protection against marine hydrodynamics, to floating structures like pontoons, which facilitate the berthing of recreational boats (Chapman and Blockley, 2009). All this leads to a decrease in the inflow and outflow of water within the marina, resulting in increased suspended sediment, organic matter, or pollutant particles, among other elements (Bulleri and Chapman, 2004; Di Franco et al., 2011; Grifoll et al., 2011) and may end up disturbing the distribution and abundance

patterns of the biota inhabiting these habitats (Small and Nicholls, 2003; Bulleri, 2007).

The surface of these new habitats, such as floating pontoons, is colonized by several benthic organisms that are part of the fouling community (Bulleri and Chapman, 2010). The fouling biota constitutes one of the major sources of diversity in harbours and marinas, since it is usually composed of a number of species of macroalgae and invertebrates (Minchin, 2007; Bulleri and Chapman, 2010) as well as certain arborescent bryozoans and hydroids (Guerra-García et al., 2015). Fouling communities are also characterized by the presence of small mobile or interstitial epifauna associated to them (Lord et al., 2015), and which also represent a key element in marine trophic webs (Bradshaw et al., 2003).

Indeed, most studies on the epifauna associated with fouling communities in marinas are not only highly fragmented, but also mainly focused on peracarid crustaceans (Minchin et al., 2012; Guerra-García et al., 2015; Ros et al., 2015). However, other taxa such as polychaete annelids also show a high ability to colonize new environments and use recreational boats as a transport vector for their expansion (Zenetos et al., 2010), constituting an important part of the macrofauna associated with the fouling in marinas. In general terms, polychaetes are one of the most frequent and abundant marine benthic organisms in artificial structures like harbours (Karalis et al., 2003). Their diverse feeding range coupled with a high diversity of reproductive strategies

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result in different responses to disturbances in urbanized areas depending on the species (Metcalf and Glasby, 2008). In fact, small-sized polychaetes have been categorized as a second-order opportunistic species, since their presence and abundance increase in disturbed conditions (Borja et al., 2000). All these characteristics make polychaetes one of the most useful taxa for detecting the impact of pollutants of anthropogenic origin (Giangrande et al., 2005) and consequently have been considered to be excellent bioindicators of marine environmental quality (Giménez-Casaldueiro et al., 2001; Dean, 2008; Sivadas et al., 2010). For instance, different families of polychaetes have shown the potential to accumulate bioavailable heavy metals from benthic habitats (Rainbow, 1995) or can tolerate high levels of organic enrichment in soft bottoms (Tomassetti and Porrello, 2005). Additionally, certain polychaetes also influence nutrient cycling between the sediment and the water column (Pocklington and Wells, 1992; Surugiu, 2005).

Nevertheless, in spite of the important role played by these organisms in urbanized environments, epifaunal polychaetes living on fouling organisms in marinas have been poorly studied and little is currently known about their ecological requirements in these man-made structures. Most previous studies have focused on the analysis of sedentary polychaete assemblages associated with hard artificial substrates or limited to fouling epifauna in some marinas (Terlizzi et al., 2000; Floerl and Inglis, 2003; Tovar-Hernández and Villalobos-Guerrero, 2009; Gavira-O'Neill et al., 2015, 2018). However, there are relatively few studies that explore the abundance and diversity patterns of epifaunal polychaetes along a wide latitudinal spatial scale (i.e. > 100 s of Km) and that also include the effect of a wide range of environmental variables (including those of anthropogenic origin) in the structuring of such assemblages. Therefore, here we provide the results of the first study that fully characterizes the polychaete fauna in marinas along a significant geographical area, i.e. the whole coastline of the Iberian Peninsula, including the north of Morocco.

For this study, we specifically sampled colonies of the bryozoan *Bugula neritina* (Linnaeus, 1758). This basibiont is one of the most common components of the fouling associated to marinas in the Mediterranean Sea and East Atlantic Ocean (Occhipinti Ambrogi, 1981; Ryland et al., 2011), growing from the upper limit of the infralittoral zone to a 6 m depth (Conradi et al., 2000); its associated epifaunal assemblage has been previously studied by Ros et al. (2015). Because of that, *B. neritina* constitutes an excellent substratum to study the polychaete assemblage present in marinas at Southwest Europe.

The main aims of this study are: (I) to describe the diversity and abundance of epifaunal polychaetes associated with *B. neritina* in marinas along the Iberian Peninsula and the north of Morocco, (II) to assess their spatial distribution patterns on these habitats, and (III) to explore the relationship of the polychaete assemblage with abiotic variables in understand which environmental factors in marinas could be more relevant to the faunal structure.

## 2. Materials and methods

### 2.1. Study area

The present study encompassed the whole coastline of the Iberian Peninsula (Spain and Portugal) and the northern coasts of Morocco. The Iberian Peninsula is located in the southwest of Europe and its coasts are washed by the Mediterranean Sea on the eastern side and the Atlantic Ocean on the northern and western side, both converging at the Strait of Gibraltar. The studied area in the north of Morocco is circumscribed to the surroundings of the Strait of Gibraltar (Fig. 1).

### 2.2. Sampling survey

The survey was carried out from May to June 2011 (late spring and early summer). Three colonies of *B. neritina* were collected from

different floating pontoons at each marina, close to the water surface and directly removed by hand. Just after collection, samples were preserved in 90% ethanol (Ros et al., 2015). In the laboratory, each colony of *B. neritina* was washed using a sieve with a mesh size of 0.5 mm with the objective of separating the macrofauna. Subsequently, each *B. neritina* frond was thoroughly inspected to ensure that all fauna was removed (Fernández-Romero et al., 2017). Polychaetes were later identified to the species level whenever possible, using a stereomicroscope and an optic microscope. Valid names and the current taxonomic position of each species followed the World Marine Species Register database (WoRMS Editorial Board, 2017). The volume of the *B. neritina* clumps was estimated by placing each sample in a graduated cylinder with a fixed amount of water and measuring volume displacement. Although we tried to collect *Bugula* colonies of the same size in all the stations, the abundance of each polychaete species was expressed as the number of individuals per 1000 ml of substrate to standardise and to eliminate the effect of potential differences in size among replicates (Ros et al., 2013; Guerra-García et al., 2015).

### 2.3. Environmental variables

The values of environmental variables are those provided by Ros et al. (2015). Three random measurements of salinity, temperature and turbidity were made in situ in each marina. Salinity and temperature (°C) were measured using a conductivity meter CRISON MM40 and turbidity, in nephelometric turbidity units (ntu), using a turbidimeter WTW 335 IR. Additionally, three surface seawater samples were collected from each marina and kept cold until being processed in the laboratory. Analysis of P, Cd, Cr, As, Cu, Ni, S, Zn and Pb content was performed by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES Varian ICP 720-ES axially viewed) after filtration through Nylon filters (pore size = 0.45 µm) and acidification with 2% HNO<sub>3</sub> (30%). Analysis of metal concentrations in water samples was performed by ICP-OES (Varian ICP 720-ES) equipped with ultrasonic nebulizer CETAC U5000AT+ after filtration through Nylon filters (pore size = 0.45 µm) and acidification with 2% HNO<sub>3</sub> (30%). Devices were calibrated using blank and standard solutions. In order to measure the concentration of heavy metals in water, a typical set of standard calibration curves with good linear regression and better relative standard deviations was achieved. All reagents used were of analytical grade or better. For preparation of standards we used < 18 MΩ/cm ultrapure water supplied from a Milli-Q Millipore system (Bedford, MA, USA) and Tracepure™ HNO<sub>3</sub> from Merck (Darmstadt, Germany). Calibration and Quality Control (QC) solutions were prepared from an ICP multi-element standard solution IV Certipur obtained from Merck and Spectrascan certified reference solution from LGC Standards GmbH (Wesel, Germany). To prevent sample contamination with traces of any metal, all material used for sample storing and treatments and all lab-ware was soaked in 2% v/v HNO<sub>3</sub> solution followed by two washes with Milli-Q water. The calibration blank was prepared with 2% v/v HNO<sub>3</sub>. Analytical blanks and standard reference materials were run in the same way as samples. The accuracy of the analytical methods was assessed through reference water sample: TR-434 Trace of metals in drinking water from INTER 2000 Program and BCR 505 No. 048 Trace Elements in Estuarine Water (CRM, 2018). The recoveries were 89.2–109.4% for all of the metals. The differences in metal concentrations between analyzed and certified values were generally < 10%. The carbon and nitrogen analyses were performed on a total organic carbon (TOC) auto analyzer (Shimadzu TOC-VCSH) with an attached total nitrogen measuring unit (TNM-1) after filtration through Whatman paper (pore diameter = 11 µm). The sample was injected into the analyzer using an auto sampler (Shimadzu ASI-V). These analyses were done in the Institute of Natural Resources and Agrobiological of Seville (IRNAS).

To examine the potential influence of human disturbance on the distribution of fouling polychaetes, human population density (mean number of people per km<sup>2</sup>) was included in the analyses. Census data

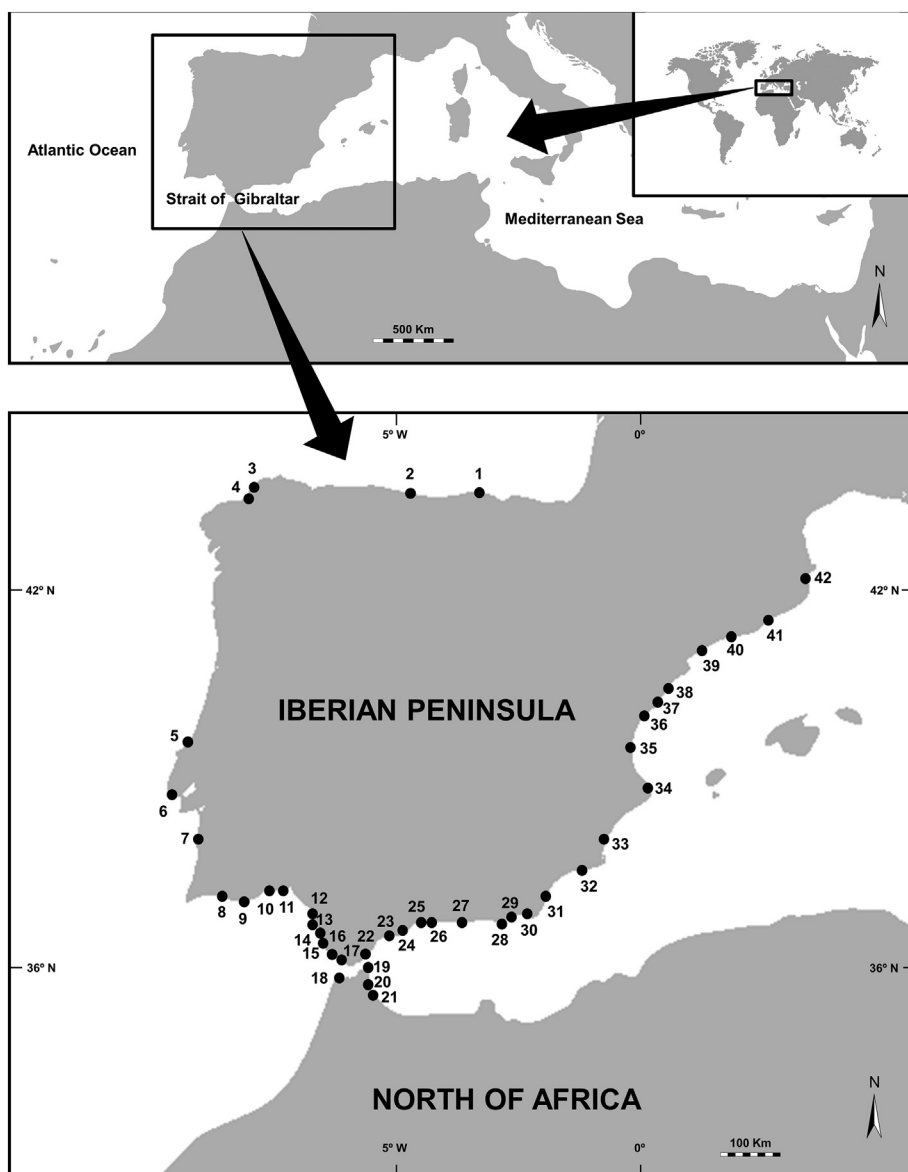


Fig. 1. Study area showing the sampling stations: Santander (1), Gijón (2), A Graña, A Coruña (3), La Marina, A Coruña (4), Nazaré (5), Cascais (6), Sines (7), Albufeira (8), Faro (9), Isla Cristina (10), El Rompido (11), Chipiona (12), Rota (13), Puerto América (14), Sancti Petri (15), Conil (16), Barbate (17), Tánger (18), Ceuta (19), Marina Smir (20), MDiq (21), La Línea (22), Fuengirola (23), Benalmádena (24), Málaga (25), La Caleta (26), Motril (27), Almerimar (28), Roquetas de Mar (29), Almería (30), Carboneras (31), Torreveja (32), Alicante (33), Denia (34), Valencia (35), Borriana (36), Oropesa (37), Benicarló (38), Tarragona (39), Vilanova (40), Barcelona (41), L'Estartit (42).

from 2011 for the locality to which each marina belongs was obtained from the National Statistical Systems of Spain ([www.ine.es](http://www.ine.es)), Portugal ([www.ine.pt](http://www.ine.pt)) and Morocco ([www.hcp.ma](http://www.hcp.ma)). The number of marina berths was also considered in the general data matrix to explore the potential influence of the primary habitat size (artificial submerged structures provided by marinas). Data was obtained from the FEAPDT (Federación Española de Puertos Deportivos y Turísticos: [www.feapdt.es](http://www.feapdt.es)) and the IPTM (Instituto Português e dos Transportes Marítimos: [www.imarpor.pt](http://www.imarpor.pt)). To investigate if the availability of host substratum affects the occurrence and abundance of fouling polychaetes, the frequency of *B. neritina* occurrence was also included. This measurement was taken following the same procedure as Ros et al. (2013). Subsequently, data was transformed using a semiquantitative scale in which 1 includes frequencies of occurrence until 20%, 2 (> 20–40%), 3 (> 40–60%), 4 (> 60–80%) and 5 (> 80–100%) according to Ros et al. (2015). The 16 environmental variables measured at each marina are represented in Table 1 (see Ros et al., 2015 for details).

#### 2.4. Data analyses

In order to determine the structure of polychaete communities in the studied habitat, the following ecological indices were used: total

number of species (S), total abundance (N), diversity expressed as Shannon-Wiener index ( $H'$ ,  $\log_2$ ; Shannon and Weaver, 1963), Pielou's evenness index ( $J'$ ), and Soyer's frequency index (F); these indices were applied to the presence and abundance of the species when it corresponded.

BIO-ENV analysis (PRIMER package; Clarke and Gorley, 2006) was used to identify the environmental factors that showed a greater correlation with the composition of polychaete assemblages. BIO-ENV compares the biological similarity matrix (based on species abundance matrix and the Bray-Curtis index) and the abiotic matrix (based on the 16 environmental variables measured in each marina and the Euclidean distances). Prior to analysis, biotic data was transformed by square root transformation, while abiotic data was first normalized and then transformed by  $\log(x + 1)$ . The relationships between environmental variables and the polychaete assemblage were studied by a Redundancy Analysis (RDA), based on their Euclidean distances and using the programming language R v.3.3.2 (R Development Core Team, 2016). For this analysis, the abiotic data matrix was standardized and the biotic data matrix was transformed by the Hellinger transformation. Prior to the Redundancy Analysis, a Detrended Correspondence Analysis (DCA) was carried out in order to identify if the data matrix responds to a linear or unimodal model.

**Table 1**  
 Sampling locations, coordinates and environmental variables measured at each location. Temp.: temperature; Turb.: turbidity; Freq.: frequency. Human population density is measured as mean number of people per km<sup>2</sup>.  
 Extracted and modified from Ros et al., 2015.

Locality	Latitude, longitude	Salinity (psu)	Temp. (°C)	Turb. (ntu)	TOC (mg/L)	N (mg/L)	P (mg/L)	S (mg/L)	Cd (µg/L)	Cr (µg/L)	As (µg/L)	Cu (µg/L)	Zn (µg/L)	Pb (µg/L)	Freq. host substrate	N° marina berths	Population density	Polychaeta present	
Atlantic Ocean																			
1. Santander	43.45° N, 3.82° W	34.27	18.43	0.80	1.5	<0.1	0.10	1248.61	2.41	<2.00	<0.05	15.05	19.45	<9.00	2	900	5176	✓	
2. Gijón	43.54° N, 5.67° W	35.57	16.97	0.33	1.6	<0.1	0.14	1282.20	0.79	<2.00	<0.05	11.60	<8.00	<9.00	2	779	1527	✓	
3. A Graña	43.48° N, 8.26° W	34.57	16.17	0.89	0	<0.1	0.13	1196.00	1.47	<2.00	<0.05	11.7	<8.00	<9.00	2	250	883	✓	
4. La Marina	43.37° N, 8.40° W	35.67	15.97	0.37	3.7	<0.1	0.13	1315.91	2.07	2.76	<0.05	10.65	12.66	<9.00	3	700	6503	✓	
5. Nazaré	39.59° N, 9.07° W	35.83	18.43	0.85	0.9	<0.1	0.11	1302.24	1.29	<2.00	<0.05	11.05	10.47	<9.00	3	52	180	✓	
6. Cascais	38.69° N, 9.42° W	34.40	19.53	0.66	1.6	<0.1	0.17	1245.10	3.25	<2.00	<0.05	7.82	10.13	<9.00	4	650	1832	✓	
7. Sines	37.95° N, 8.87° W	36.27	17.63	0.55	1.7	<0.1	0.14	1317.39	<0.70	2.41	<0.05	9.87	<8.00	<9.00	3	230	67	✓	
8. Albufeira	37.08° N, 8.27° W	35.80	19.87	0.34	0.9	<0.1	0.10	1303.83	1.58	2.05	<0.05	9.83	13.96	<9.00	3	475	251	✓	
9. Faro	37.01° N, 7.94° W	36.33	21.03	0.62	1	<0.1	0.16	1309.44	2.82	<2.00	<0.05	13.93	9.45	<9.00	2	300	289	✓	
10. Isla Cristina	37.19° N, 7.34° W	36.07	23.00	6.98	1.1	<0.1	0.10	1311.73	2.34	2.41	<0.05	16.25	16.02	<9.00	2	231	448	–	
11. El Rompido	37.22° N, 7.13° W	35.53	23.00	10.35	29	<0.1	0.17	1293.27	1.43	<2.00	<0.05	11.46	22.99	<9.00	2	387	85	✓	
12. Chupiona	36.74° N, 6.43° W	35.37	19.27	10.05	2.1	1.10	0.13	1254.72	1.47	<2.00	<0.05	9.2	<8.00	<9.00	2	447	573	✓	
13. Rota	36.62° N, 6.35° W	36.37	19.53	10.11	2	<0.1	0.14	1290.31	2.57	<2.00	<0.05	10.39	<8.00	<9.00	3	209	347	✓	
14. Puerto América	36.54° N, 6.38° W	36.30	19.90	4.12	31	<0.1	0.07	1319.93	1.09	<2.00	<0.05	9.83	<8.00	<9.00	3	319	10,154	✓	
15. Sancti Petri	36.40° N, 6.21° W	36.70	19.93	5.83	2.7	<0.1	0.10	1331.86	2.47	2.42	<0.05	7.03	<8.00	<9.00	3	94	389	–	
16. Conil	36.29° N, 6.14° W	36.67	19.57	5.97	2	<0.1	0.16	1323.53	1.15	2.02	<0.05	8.62	<8.00	<9.00	2	97	245	–	
Strait of Gibraltar																			
17. Barbate	36.19° N, 5.93° W	35.67	19.73	10.21	2.1	<0.1	0.12	1206.51	0.96	<2.00	<0.05	14.12	<8.00	<9.00	2	314	160	✓	
18. Tánger	35.79° N, 5.81° W	36.03	19.37	1.26	3.0	1.0	0.03	1438.09	3.12	19.28	5.74	9.11	33.31	12.33	2	500	229	✓	
19. Ceuta	35.89° N, 5.32° W	36.13	21.87	1.43	2.7	1.0	<0.03	1416.79	1.46	14.57	7.5	20	41.46	<9.00	2	325	4229	✓	
20. Marina Smir	35.75° N, 5.34° W	36.37	20.23	1.06	3.6	1.0	<0.03	1451.69	2.08	14.73	<0.05	<7.00	9.92	<9.00	3	450	283	✓	
21. M'Diq	35.68° N, 5.31° W	36.20	21.37	0.70	3.0	1.0	<0.03	1465.83	2.64	21.36	4.61	11.29	15.18	9.37	2	120	283	–	
22. La Línea	36.16° N, 5.36° W	36.87	18.60	1.28	5.9	<0.1	0.11	1322.06	<0.70	<2.00	<0.05	9.4	<8.00	<9.00	3	624	3370	✓	
Mediterranean Sea																			
23. Fuengirola	36.54° N, 4.62° W	36.40	20.77	0.5	1.9	<0.1	0.03	1463.47	1.47	21.90	<0.05	<7.00	9.35	<9.00	2	275	7145	✓	
24. Benalmádena	36.60° N, 4.51° W	36.40	20.47	1.26	2.1	<0.1	<0.03	1444.34	2.28	12.22	0.77	16.65	16.83	<9.00	2	1140	2373	✓	
25. Málaga	36.72° N, 4.41° W	36.40	24.00	1.49	2.9	1.0	<0.03	1474.08	2.47	18.74	15.54	15.51	47.27	9.32	4	107	1437	✓	
26. La Caleta	36.75° N, 4.07° W	36.53	24.33	3.24	4.0	1.0	<0.03	1479.64	2.36	16.59	<0.05	18.77	27.64	10.20	1	277	488	✓	
27. Motril	36.72° N, 3.53° W	34.97	25.07	1.91	3.0	1.0	<0.03	1286.44	1.33	9.49	8.16	15.94	14.61	20.37	3	193	555	–	
28. Almerimar	36.70° N, 2.79° W	36.87	26.47	1.21	3.0	1.0	<0.03	1460.74	<0.70	13.11	<0.05	8.88	10.72	13.20	1	1100	371	–	
29. Roquetas de Mar	36.76° N, 2.61° W	36.90	26.13	1.25	4.9	1.0	<0.03	1439.57	2.22	14.59	<0.05	10.04	14.66	<9.00	1	237	1506	✓	
30. Almería	36.83° N, 2.46° W	36.77	25.70	0.92	19.6	1.0	<0.03	1417.35	1.51	12.66	<0.05	8.06	12.17	27.47	2	277	643	✓	
31. Carbonera	36.99° N, 1.90° W	37.90	25.77	0.97	6.5	1.0	0.03	1476.06	0.84	20.20	25.31	8.02	14.01	14.00	1	48	86	–	
32. Torreveja	37.97° N, 0.68° W	37.90	27.93	1.23	4.8	1.0	<0.03	1480.67	1.00	18.00	20.46	9.58	18.55	<9.00	2	570	1430	–	
33. Alicante	38.34° N, 0.49° W	38.23	28.07	0.43	3.1	1.0	<0.03	1485.28	2.57	17.77	15.61	11.13	23.09	<9.00	4	400	1661	✓	
34. Dénia	38.85° N, 0.11° W	37.03	26.70	0.69	3.1	3.3	<0.03	1299.61	1.42	13.33	16.26	11.80	17.01	<9.00	3	300	676	✓	
35. Valencia	39.43° N, 0.33° W	37.77	27.80	2.55	4.3	<0.1	0.04	1446.34	1.30	20.25	36.31	22.16	83.89	<9.00	3	206	5928	–	
36. Borraina	39.86° N, 0.07° W	37.70	26.37	1.00	5.5	1.1	<0.03	1445.69	3.51	17.66	25.51	10.98	10.02	23.78	3	713	126	✓	
37. Orpesa	40.08° N, 0.13° E	37.90	25.37	0.53	3.7	1.0	<0.03	1525.35	2.90	13.00	<0.05	14.33	19.97	<9.00	1	668	126	–	
38. Benicarló	40.42° N, 0.43° E	36.87	26.00	0.46	7.1	1.7	0.07	1508.07	1.44	19.83	<0.05	9.86	8.59	<9.00	3	293	126	✓	
39. Tarragona	41.11° N, 1.25° E	38.00	25.83	0.46	4.3	1.0	0.04	1507.00	<0.70	12.20	<0.05	23.99	59.68	17.02	3	441	2436	✓	
40. Vilanova	41.21° N, 1.73° E	37.80	24.43	0.98	5.1	1.0	<0.03	1441.47	2.18	14.51	<0.05	14.46	16.47	<9.00	4	812	1976	–	
41. Barcelona	41.38° N, 2.18° E	37.80	23.77	1.27	5.3	1.0	0.51	1514.66	1.01	14.51	15.62	17.30	36.49	51.85	5	200	16,449	✓	
42. L'Estretit	42.05° N, 3.21° E	36.60	22.20	0.67	3.9	1.0	0.04	1718.47	1.18	18.57	8.69	19.79	26.95	22.81	2	738	172	✓	

The occurrence and abundance of dominant polychaete species were analyzed using Generalized Linear Models (GLM; McCullagh and Nelder, 1989) by programming language R v.3.3.2. The occurrence component was modeled through a Binomial distribution (logit link). On the other hand, a Negative Binomial (log link) was carried out with the aim of removing the possible overdispersion present in the abundance models; this only considered samples with species densities above zero and taking discrete values from the data matrix (Stefánsson, 1996; Fletcher et al., 2005). Both analyses included the same set of independent variables. To avoid similar models, the existence of collinearity between two environmental variables was considered when these factors showed a Pearson correlation coefficient ( $r$ ) > 0.6. Consequently, four predictor variables were excluded from the models: Salinity and Cr (correlated with temperature), S (correlated with Cr and temperature) and Cu (correlated with Zn).

Model selection was carried out based on second order Akaike information criterion (AICc) for small sample sizes (AICc, Burnham and Anderson, 2002). For the present study, we assumed a significant binomial model for those that had < 2 AICc difference with respect to the model that presented the smallest AICc. The Akaike weight ( $W_i$ ) was calculated for each one of these binomial models. The predictor variables were ranked in order of magnitude based on the combination of Akaike Weight ( $\Sigma W_i$ ) of all the significant presence/absence models that contained the same variable. Model averaged estimators were used to improve the precision and reduce bias of the analyses compared with single estimator from the selected 'best' model (Burnham and Anderson, 2002). Consequently, the coefficients of each predictor variable were determined based on the most significant models average with a 95% confidence interval. Alternatively, the ten models with the lowest AICc were selected for the negative binomial models. The predictor variables were ranked in order of magnitude of the estimated coefficient of each factor ( $\beta$ ). Because each of the abundance models has a different dispersion parameter ( $1/k$ ), the estimated coefficients of each predictor variable were represented in a range of maximum and minimum values registered in such models, with a 95% confidence interval. The predictor variables were standardized in order to compare the magnitude effect of each estimated coefficient for each model. In addition, to estimate the variability of the data explained by each model, deviation ( $D^2$ ) was also calculated.

To select more reliable models, interaction terms and non-linear terms (i.e. quadratics terms of each predictor variable) were also included in the set of variables (Wood, 2006). For further analysis in the present study, the term "quadratic function" refers to the combination of the quadratic term of one predictor variable together with the original variable ( $x^2 + x$ ). To prevent risks of overfitting, higher-order polynomials were not used for the predictor variables. The performance of the significant Binomial models-average was carried out by evaluating the area under the curve (AUC). In the case of Negative Binomial models, a Pearson correlation between predicted and observed values was calculated to assess the performance for each model.

To ensure that the values of variables sampled in nearby locations are independent of each other, a spatial autocorrelation analysis in the model's residuals was tested using the global Moran's I test (Dormann et al., 2007).

### 3. Results

#### 3.1. Spatial patterns of polychaete diversity and abundance

In the present study, a total of 295 polychaete individuals corresponding to 32 species or morphospecies were found, belonging to 14 families (Table 2). Some specimens were only identified to the genus or family level, due to poor condition of preservation or lack of some diagnostic features.

The families best represented overall in terms of number of species and abundance were Syllidae, Cirratulidae and Sabellidae, comprising a

combined 59.4% of the total number of species and 76.8% of total polychaete abundance. Syllidae was the most diverse family, with 13 different species. The numerically dominant species were the syllid *Salvatoria clavata* (40% of total specimens) followed by the cirratulid *Ctenodrilus serratus* (11%), the sabellid *Amphiglena mediterranea* (10.5%), and the nereidid *Platynereis dumerilii* (6%). According to Soyer's frequency index (F), only one out of 32 species found can be classified as constant ( $F \geq 50$ ), one as common ( $F$  between 25 and 49), and the remaining 30 species as rare ( $F < 25$ ). Thus, *S. clavata* was constant (frequency score: 70.9%) and *P. dumerilii* was common (32.26%).

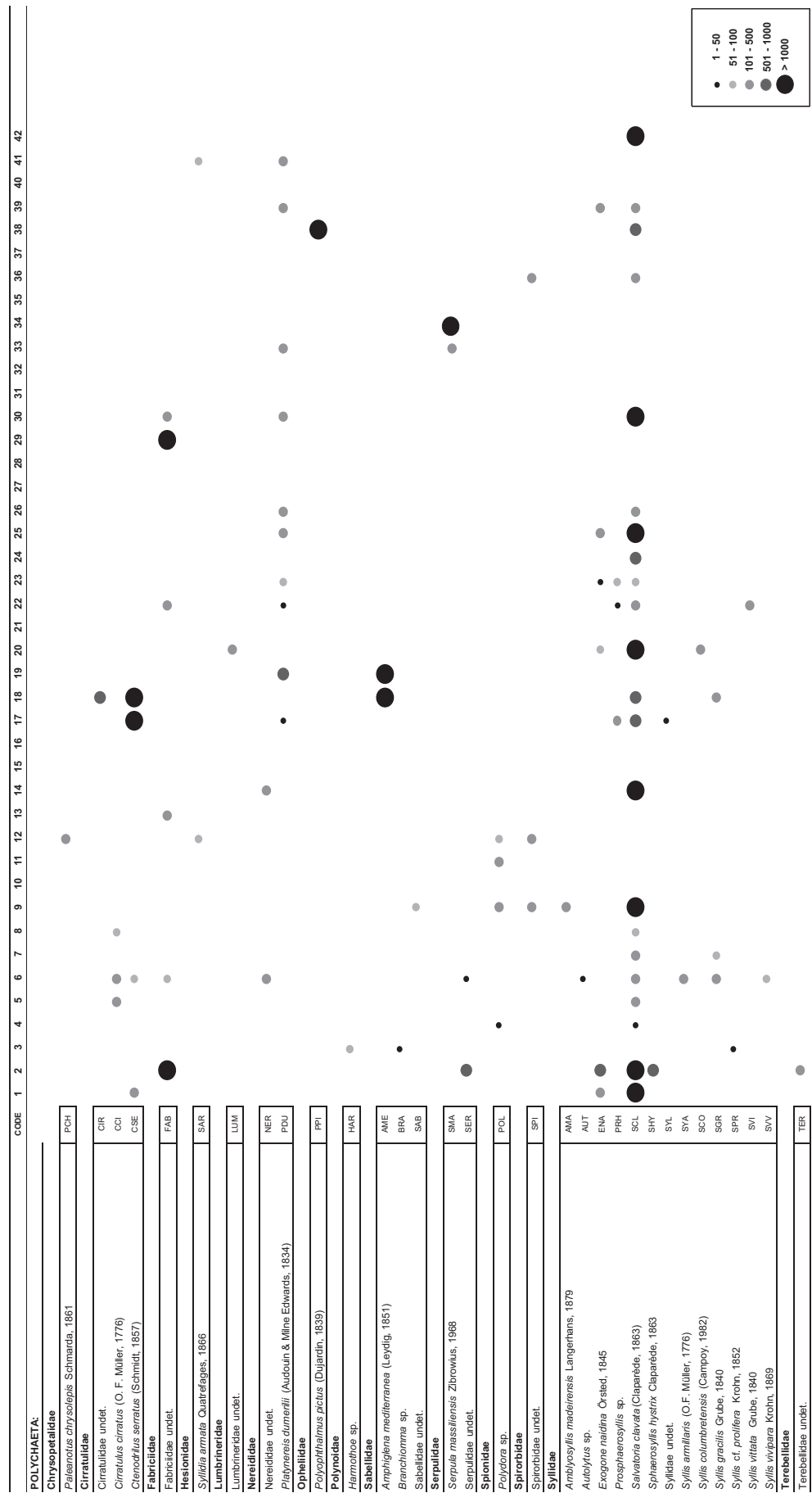
The total number of species per station and mean number of species per replicate, abundance, diversity and evenness on each marina are presented in Fig. 2. When considering the total number of polychaete species in each marina, the highest value (10 species) was found on Cascais (marina 6) and the lowest (one species) on El Rompido (11), Rota (13), Benalmádena (24), Roquetas de Mar (29), Dénia (34) and L'Estartit (42). The highest abundances were measured in Ceuta (19) and Tángier (18) with 16,000 and 10,000 ind. per 1000 ml of substrate respectively, while the lowest abundance was detected in A Graña (4) with 57 ind. per 1000 ml of substrate. The Shannon-Wiener diversity index showed greater values in marinas located in the Atlantic and the Strait of Gibraltar than in those from the Mediterranean Sea. However, values of evenness were rather similar in the Atlantic and the Mediterranean while slightly lower values were detected in the Strait of Gibraltar.

Polychaetes were roughly distributed along the whole studied area. Abundance values higher than 5000 individuals per 1000 ml of substrate were detected only in marinas 2, 18 and 19, while for other marinas abundance ranged between 500 and 5000 ind. per 1000 ml of substrate. The numerically dominant *S. clavata* was found in 22 out of the 42 sampled marinas. Its abundance and distribution displayed a similar pattern to that of the whole polychaete assemblage, with abundance values ranging between 1001 and 5000 individuals per 1000 ml of substrate in most marinas across all the studied regions (Fig. 3).

Prior to the Redundancy Analysis (RDA), a Detrended Correspondence Analysis (DCA) was carried out, which was used to identify whether the data corresponds to a direct gradient. A value < 3 was obtained for the first axis length of the DCA and therefore the data responds to a linear model (Ter Braak and Šmilauer, 1998), which recommends performing an RDA (Fig. 4). The first axis absorbed 44.40% of the total variance and was significantly correlated with temperature. The second axis (17.79%) was significantly correlated with turbidity, frequency of host substrate, N and Zn concentration whereas the third axis (12.66%) was mainly correlated with TOC and P concentration. The global RDA analysis determined a relation between the environmental variables and the polychaete assemblage with a significance of  $p < 0.01$ .

The ordination of polychaete species in the RDA seems to be related to nutrient enrichment and values of heavy metals in the studied marinas. Thus, species such as *S. clavata*, *P. dumerilii*, *Exogone naidina*, *A. mediterranea* and *Cirratulus cirratus* were associated with marinas characterized by higher levels of N and Zn concentration, and with Cu in turn as it is highly correlated with Zn concentrations. Conversely, other taxa such as *C. serratus* or *Prosphaerosyllis* sp. were located at the negative side of axis 2 and the positive side of axis 3 and were associated with lower values of N, Zn, P and TOC concentration (Fig. 4; Table 3). However, other species do not fit this pattern, such as *Polydora* sp., which is related to organic contamination and certain nutrients such as P, whereas in marinas with higher values of N and Zn this taxon was not detected. On the other hand, species that were only found in a single marina were clustered closer to the origin of coordinates; these were not plotted in the RDA for the sake of clarity. Finally, the BIO-ENV showed that the combination of variables that best explained polychaete distribution was that obtained for turbidity, N, P and frequency

**Table 2**  
List of polychaete species found and abundance (ind./1000 ml substrate) on *B. neritina* samples at each marinas.



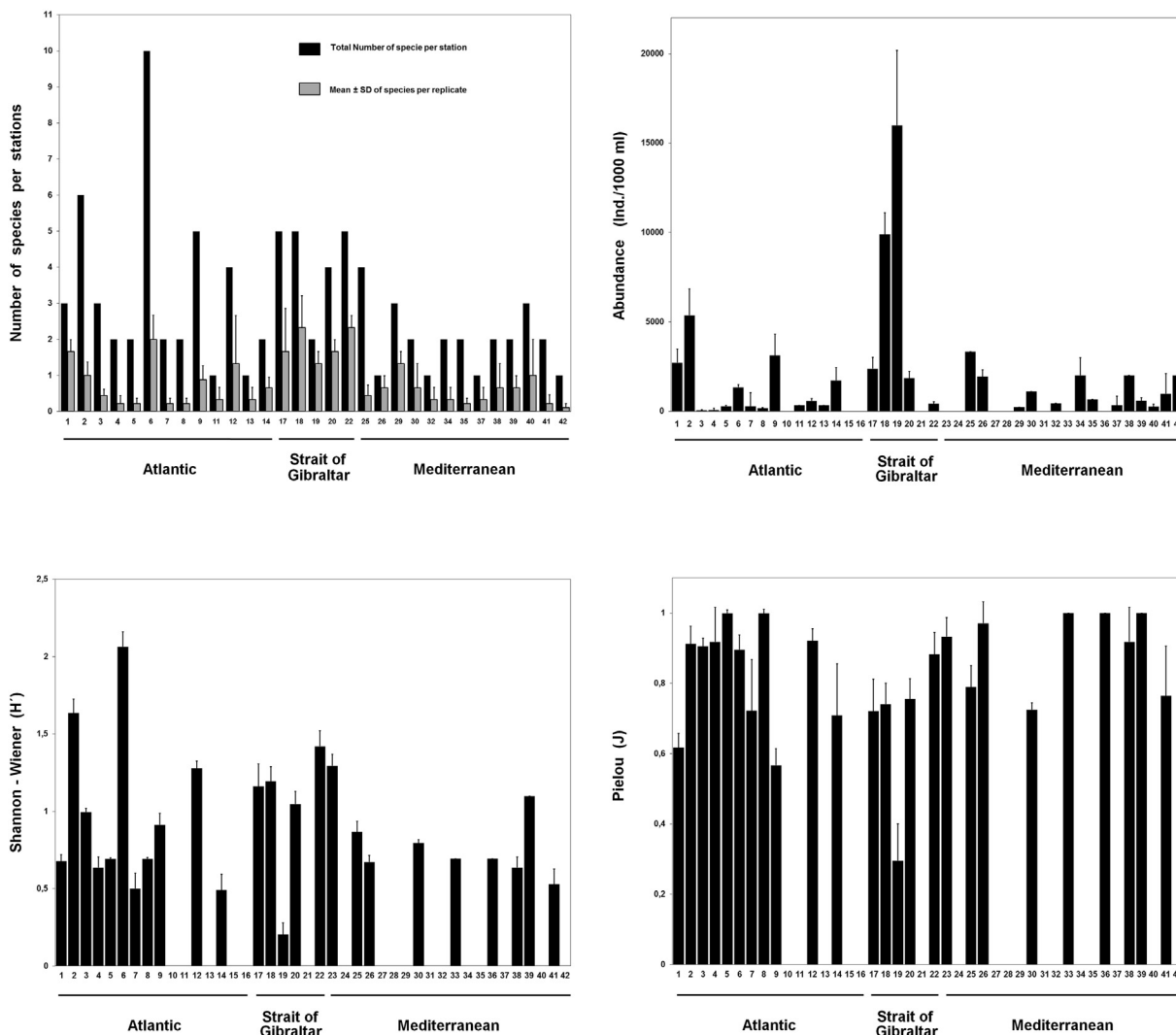


Fig. 2. Descriptive indices of the polychaete assemblage measured in each marina. Number of species is expressed as Number of total species per station and also as mean ± SD of number of species per replicate (n = 3). Abundance, Shannon-Wiener diversity and Pielou evenness are expressed as mean ± SD of the three replicates.

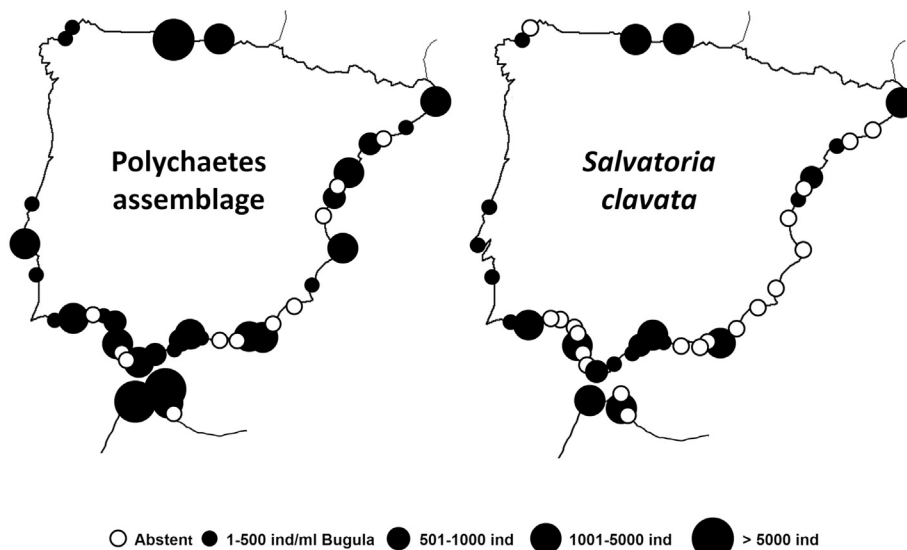


Fig. 3. Abundance values of polychaetes associated with *B. neritina* in sampled marinas (ind./1000 ml of substrate). Left showing abundances of the whole polychaete assemblage. Right showing abundances of *S. clavata*.

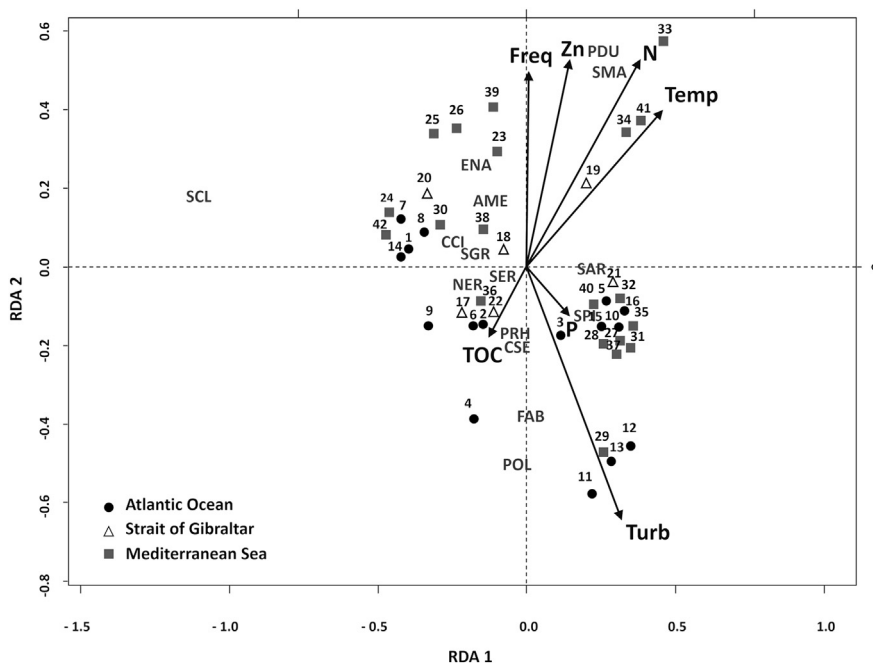


Fig. 4. Graph representation of the stations and polychaete species with respect to the first two axes of the Redundancy Analysis (RDA). The acronyms in the RDA plot refer to the name of each polychaete species (see CODE in Table 2).

Table 3  
Summary of the results of the RDA analysis.

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.061	0.024	0.017
Percentage of species variance	44.40	17.79	12.66
Cumulative Proportion	44.40	62.19	74.86
Correlation with environmental variables			
Temperature (°C)	0.633***	0.422**	–
Turbidity (ntu)	0.387**	–0.758***	–
N (mg/L)	0.521***	0.539***	0.415**
Zn (µg/L)	–	0.522***	–0.431**
Freq. host substrate	–	0.517***	–0.297*
P (mg/L)	–	–	–0.744***
TOC (mg/L)	–	–	–0.388**

See also Fig. 4.

Permutation test for all constrained eigenvalues.  
Pseudo-F: 1.533115 (with 7, 34 Degrees of Freedom).  
Significance: 0.00699 < 0.01.

- \* p < 0.05.
- \*\* p < 0.01.
- \*\*\* p < 0.001.

of substrate (r = 0.350, p < 0.05).

### 3.2. Environmental variables involved in the occurrence and abundance of *S. clavata*

Two Generalized Linear Models were carried out to explore the environmental variables related to the presence and abundance of *S. clavata* in marinas along the studied coasts. First, the presence/absence component was modeled through a Binomial distribution and secondly, the abundance component was modeled with a Negative Binomial distribution.

Temperature<sup>2</sup> and turbidity were the most important factors explaining the occurrence of *S. clavata* along the Iberian Peninsula and the north of Morocco. The presence of this species was related to temperature<sup>2</sup> reaching a maximum value around 22 °C. For higher or lower values of temperature, the probability of *S. clavata* presence tends to decrease (Fig. 5a). Its occurrence was negatively related with turbidity

(Fig. 5b). Although the best presence/absence model had these environmental factors as explanatory variables, the first ten additional models with a  $\Delta i$  lower than 2 were also considered significant (Table 4a:  $\Delta i < 2$ ). These models also included temperature, TOC and P along with the non-linear terms TOC<sup>2</sup> and P<sup>2</sup> (all the interaction terms were not supported by the significant models). The variability explained by significant models ranged from 22.7% to 30% (Table 4a: D2). Model averaged estimated coefficients (Table 4b) showed that the only significant relations were with turbidity, temperature<sup>2</sup> and temperature. All the estimate coefficients of these predictor variables were negatively related to the presence of *S. clavata* ( $\beta = -1.08$ , p < 0.05;  $\beta = -1.27$ , p < 0.01 and  $\beta = -1.21$ , p < 0.01 respectively). Both turbidity and the quadratic function of temperature (Temp.<sup>2</sup> + Temp.) were the most important variables for explaining its occurrence ( $\Sigma w_i = 1$  for both cases; Table 4b) followed by the quadratic function of TOC ( $\Sigma w_i = 0.485$ ) and the quadratic function of P ( $\Sigma w_i = 0.328$ ). The average performance of the best significant models was good (AUC = 0.82).

When the abundance of *S. clavata* (conditioned by presence) was the response variable, the ten significant models selected, which explained from 62.7% to 70.8% of variability, included a total of ten explanatory variables (Table 5a: D<sup>2</sup>). These factors were the non-linear terms: temperature<sup>2</sup>, number of marina berths<sup>2</sup>, frequency of host substrate<sup>2</sup>, TOC<sup>2</sup> and P<sup>2</sup>, in addition to the predictor variables: frequency of host substrate, TOC, N, P and As concentration. Interaction terms were also rejected for the significant abundance models. The abundance of *S. clavata* was also related to temperature<sup>2</sup>, with a similar pattern to that described for the best presence/absence model. Taking into account the remaining predictor variables, the abundance of *S. clavata* was also negatively related with frequency<sup>2</sup> and positively with number of marina berths<sup>2</sup>, N, P, TOC, As and the non-linear terms TOC<sup>2</sup> and P<sup>2</sup>. Moreover, all these variables were significantly influenced in the abundance of *S. clavata*, with a p < 0.001, with the exception of As concentration that showed a p-value lower than 0.05. The standardized estimated coefficients ( $\beta$ ) provide a quantitative measure of the importance of their effect on the response variable. Fig. 6 shows the abundance response of *S. clavata* to different environmental pollutants i.e. N, P, TOC and As, based on the greater range of values of the



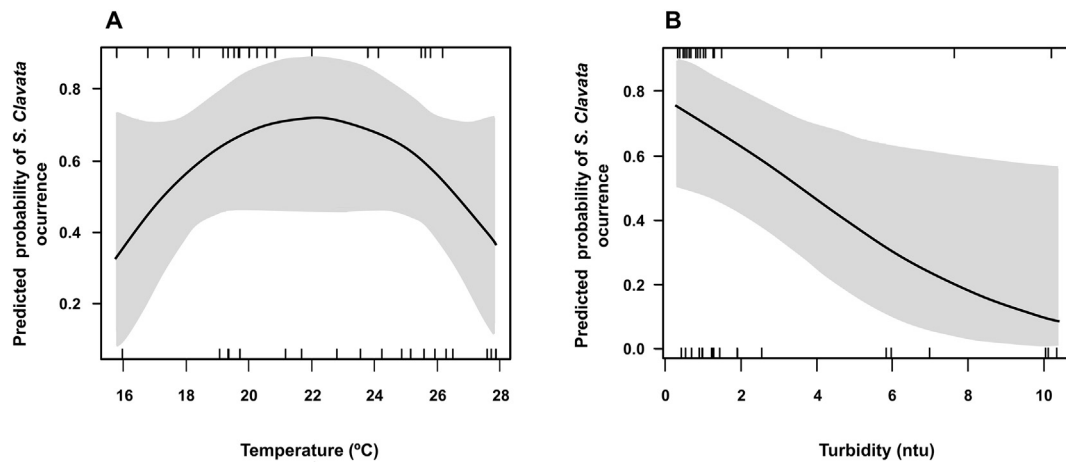


Fig. 5. GLM plots showing the predicted effect of temperature (A) and turbidity (B) on occurrence (presence/absence) of *S. clavata*. Shading indicates 95% confidence limits for the fitted relationship.

Table 4

Models selection results explaining occurrence of *S. clavata*: (a) Akaike's Information Criterion corrected for small sample size (AICc), AICc weights ( $w_i$ ) and AICc difference between the AICc of each model and the AICc of the best fitted model ( $\Delta_i$ ) were used for comparison. Variables: Turb., turbidity; Temp., Temperature; P, phosphorus; TOC, total organic carbon. (b) Model averaged coefficients ( $\beta$ ) of explanatory variables present in the significant models for *S. clavata* occurrence. Variables were ranked in order of the sum of their Akaike weights ( $\Sigma w_i$ ). Adjusted standard error (SE) of model averaged coefficients,  $z$ -values and  $p$  values are included.

Best models	df	AICc	$\Delta_i$	$w_i$	LogLik	D <sup>2</sup>	Moran's I $\pm$ SD
a) Response <i>S. clavata</i> occurrence							
1.- Temp. <sup>2</sup> + Turb.	3	51.10	0.00	0.147	-22.26	0.234	-0.085 $\pm$ 0.003
2.- Temp. <sup>2</sup> + Turb. + TOC	4	51.36	0.26	0.129	-21.16	0.272	-0.081 $\pm$ 0.003
3.- Temp. <sup>2</sup> + Turb.	3	51.49	0.39	0.121	-22.46	0.227	-0.082 $\pm$ 0.003
4.- Temp. <sup>2</sup> + Turb. + TOC	4	51.75	0.65	0.106	-21.36	0.265	-0.077 $\pm$ 0.003
5.- Temp. <sup>2</sup> + Turb. + P <sup>2</sup>	4	51.97	0.87	0.095	-21.47	0.261	-0.083 $\pm$ 0.003
6.- Temp. <sup>2</sup> + Turb. + TOC <sup>2</sup>	4	52.03	0.93	0.092	-21.50	0.260	-0.082 $\pm$ 0.003
7.- Temp. <sup>2</sup> + Turb. + TOC + P <sup>2</sup>	5	52.30	1.20	0.081	-20.34	0.300	-0.079 $\pm$ 0.003
8.- Temp. <sup>2</sup> + Turb. + P	4	52.38	1.28	0.077	-21.68	0.254	-0.085 $\pm$ 0.003
9.- Temp. <sup>2</sup> + Turb. + TOC <sup>2</sup>	4	52.40	1.30	0.077	-21.69	0.254	-0.079 $\pm$ 0.003
10.- Temp. <sup>2</sup> + Turb. + P <sup>2</sup>	4	52.44	1.34	0.075	-21.70	0.253	-0.081 $\pm$ 0.003
Predictor	$\beta$	Adjusted SE	$z$	$P$	$\Sigma w_i^a$		
b) <i>S. clavata</i> occurrence							
Intercept	0.14	0.41	0.36	0.720			
Turb.	-1.08	0.47	-2.07	0.023*	1		
Temp. <sup>2</sup>	-1.27	0.47	-2.29	0.006**	0.621		
Temp.	-1.21	0.45	-2.67	0.008**	0.379		
TOC	0.67	0.49	1.37	0.171	0.316		
P <sup>2</sup>	-0.88	1.69	-0.52	0.604	0.251		
TOC <sup>2</sup>	0.56	0.49	1.14	0.256	0.169		
P	-0.48	0.56	-0.85	0.394	0.077		

<sup>a</sup>  $\Sigma w_i$  values are recalculated considering only the models with  $\Delta AICc < 2$ .

\* Significant coefficient value  $p < 0.05$ .

\*\* Significant coefficient value  $p < 0.01$ .

estimated coefficient registered in the negative binomial models (Table 5b). The performance of the ten best significant models was high ( $r = 0.77$ ;  $p < 0.01 - r = 0.84$ ;  $p < 0.01$ ).

### 3.3. Spatial autocorrelation

In order to determine if the response variables measured in the marinas were totally independent of each other, a spatial autocorrelation analysis was carried out using I of Moran index. The previous analysis revealed that neither the initial dataset of the presence/absence nor the abundance of *S. clavata* were spatially autocorrelated. Examination of the best fitted occurrence and abundance model residuals for this species showed that they were not spatially autocorrelated (Tables 4a; 5a). Hence, we assume that the sampled marinas

were spatially independent of each other and therefore it was not necessary to include any residual spatial autocorrelation modeling in the presence/absence and abundance models.

### 4. Discussion

Several studies have explored the distribution patterns and structural response of polychaete assemblages to environmental pollutants in anthropogenic disturbance areas (e. g. Elías et al., 2006; Gillet et al., 2008; Jaubet et al., 2011). However, in many cases the spatial replication is insufficient to fully assess such impact. Furthermore, although the effects of pollutants on benthic polychaetes have been exhaustively researched (Warwick, 2001; Dauvin, 2008; Chen et al., 2010; Cabral-Olivera et al., 2014), studies on epibiont polychaetes in fouling

**Table 5**

Models selection results explaining abundance of *S. clavata*: (a) Akaike's Information Criterion corrected for small sample size (AICc). Dispersion parameter of each model (1/k) is also included. Variables: Turb., turbidity; Temp., Temperature; Freq., frequency of occurrence of *Bugula neritina*; Berths, number of marina berths; N, nitrogen; P, phosphorus; TOC, total organic carbon; As, arsenic. (b) Range of values of coefficients ( $\beta$ ) of explanatory variables present in the significant models for *S. clavata* abundance. Variables were ranked in order of decreasing range of values of models coefficient. Range of values of adjusted standard error (SE) of model coefficients, z-values and p values are also represented.

Best models	df	AICc	1/k	LogLik	D <sup>2</sup>	Moran's I ± SD
a) Response <i>S. clavata</i> abundance						
1.- Temp. <sup>2</sup> + Berths <sup>2</sup> + Freq. <sup>2</sup> + N + P <sup>2</sup> + TOC <sup>2</sup>	7	170.2	0.063	-71.100	0.650	-0.096 ± 0.003
2.- Temp. <sup>2</sup> + Berths <sup>2</sup> + Freq. <sup>2</sup> + N + P <sup>2</sup> + TOC	7	170.4	0.064	-71.207	0.646	-0.101 ± 0.003
3.- Temp. <sup>2</sup> + Berths <sup>2</sup> + Freq. <sup>2</sup> + N + P <sup>2</sup> + TOC <sup>2</sup>	7	171.0	0.067	-71.530	0.635	-0.103 ± 0.003
4.- Temp. <sup>2</sup> + Berths <sup>2</sup> + Freq. <sup>2</sup> + N + P <sup>2</sup> + TOC <sup>2</sup>	7	171.0	0.068	-71.507	0.635	-0.089 ± 0.003
5.- Temp. <sup>2</sup> + Berths <sup>2</sup> + Freq. <sup>2</sup> + N + P <sup>2</sup> + TOC	7	171.0	0.068	-71.524	0.635	-0.094 ± 0.003
6.- Temp. <sup>2</sup> + Berths <sup>2</sup> + Freq. <sup>2</sup> + N + P <sup>2</sup> + TOC	7	171.1	0.069	-71.575	0.633	-0.107 ± 0.003
7.- Temp. <sup>2</sup> + Berths <sup>2</sup> + Freq. <sup>2</sup> + N + P <sup>2</sup> + TOC	7	171.4	0.070	-71.730	0.627	-0.101 ± 0.003
8.- Temp. <sup>2</sup> + Berths <sup>2</sup> + Freq. <sup>2</sup> + N + P <sup>2</sup> + TOC <sup>2</sup>	7	171.5	0.071	-71.748	0.627	-0.096 ± 0.003
9.- Temp. <sup>2</sup> + Berths <sup>2</sup> + Freq. <sup>2</sup> + N + P <sup>2</sup> + TOC + As	8	172.6	0.045	-69.147	0.708	-0.085 ± 0.003
10.- Temp. <sup>2</sup> + Berths <sup>2</sup> + Freq. <sup>2</sup> + N + P <sup>2</sup> + TOC + As	8	173.0	0.046	-69.343	0.703	-0.102 ± 0.003
b) <i>S. clavata</i> abundance						
Predictor	$\beta^a$	Adjusted SE <sup>a</sup>	z <sup>a</sup>	p <sup>a</sup>		
Intercept	3.090–3.101	0.067–0.076	45.533–40.699	0.000***–0.000***		
N	0.691–0.821	0.128–0.163	5.374–5.027	0.000***–0.000***		
P	0.538–0.656	0.151–0.145	3.565–4.502	0.000***–0.000***		
P <sup>2</sup>	0.489–0.567	0.130–0.124	3.748–4.575	0.000***–0.000***		
Temp. <sup>2</sup>	-0.357–0.470	0.094–0.090	-3.769–5.195	0.000***–0.000***		
TOC	0.313–0.358	0.081–0.075	3.831–4.731	0.000***–0.000***		
TOC <sup>2</sup>	0.308–0.317	0.079–0.081	3.853–3.913	0.000***–0.000***		
Berths <sup>2</sup>	0.274–0.315	0.087–0.084	3.133–3.741	0.002**–0.000***		
Freq. <sup>2</sup>	-0.191–0.259	0.076–0.074	-2.495–3.467	0.012*–0.000***		
Freq.	-0.184–0.205	0.077–0.080	-2.375–2.569	0.017*–0.010*		
As	0.171–0.198	0.082–0.083	2.088–2.368	0.036*–0.017*		

<sup>a</sup> Range of values (min.-max.)

\* Significant coefficient value p < 0.05.

\*\* Significant coefficient value p < 0.01.

\*\*\* Significant coefficient value p < 0.001.

assemblages are still lacking. This study provides therefore the first characterization at a regional scale of the epibiont polychaete assemblage in marinas, in this case that associated with *B. neritina* along the whole Iberian Peninsula coasts including the north of Morocco.

#### 4.1. Characteristic of epibiont polychaete assemblage

Syllidae is one of the dominant polychaete families in terms of species number and abundance in many natural coastal assemblages (Çinar and Ergen, 2002; Serrano et al., 2006; Musco, 2012). On the contrary, its presence is scarce in habitats subjected to high environmental stress (Giangrande et al., 2004), such as sewage pollution. However, Frascchetti et al. (2006) found that syllids were the most abundant polychaete family in coasts affected by sewage pollution; our results showed a similar pattern in the studied marinas. Serrano et al. (2006) stated that habitat complexity and physical disturbances derived from hydrodynamism were the most relevant factors in the structuring of syllid assemblages in shallow rocky environments. Hence, it is plausible that the combination of shelter and habitat provided by *B. neritina* and the low hydrodynamism present inside marinas may somehow favour the presence of syllids (Giangrande, 1990; Musco, 2012). Thus, our study presents the first evidence where syllids are the dominant epibiont polychaete family in marine artificial structures in conditions of high levels of pollutants of anthropogenic origin.

Furthermore, there was a marked spatial heterogeneity in the distribution of syllid species across marinas. Thus, most species were only detected in marinas located on the Atlantic coast and Strait of Gibraltar, while a small number of species were also distributed along the Mediterranean stations. Although marinas are semi-enclosed systems and pollutants generated by recreational boats tend to remain confined

inside them (Chou and Jaafar, 2002), the influence of inflow water can also increase the accumulation of heavy metals and other pollutants that are harmful for benthic biota. For instance, it is well known that the Mediterranean Sea presents one of the highest pollution rates in the world, mainly in industrial areas and harbours (Bianchi and Morri, 2000; Zorita et al., 2007). Despite heavy metal values detected in the present study being similar to those present in the water column of port environments around the world, Mediterranean marinas occasionally showed heavy metal concentrations that exceeded those values recorded by other authors (e.g.: Fatoki and Mathabatha, 2001; Stakeniene et al., 2011; Sany et al., 2013; Delshab et al., 2017; Kenworthy et al., 2018). In fact, Spanish Mediterranean marinas have been recently considered to be of “very high susceptibility to pollution” (Gómez et al., 2017). Moreover, in our analysis, greater concentrations of TOC, N and heavy metals were detected in Mediterranean marinas than in the Atlantic ones (cf. Table 1). This may result in a structure change of the polychaete assemblage and eventually in impoverished diversity in the fouling biota in the studied marinas (Dhainaut-Courtois et al., 2000; Dean, 2008). In fact, there was a decrease of values of species richness and diversity (H') in the Mediterranean marinas when compared to those in the Strait of Gibraltar and Atlantic coasts.

Macrofaunal communities are subjected to continuous stress in marinas and harbours, which often result in a species richness decrease (Dhainaut-Courtois et al., 2000). This seems to be the case of the marinas studied where clumps of *B. neritina* just housed a total of 32 species along the whole considered area. Nevertheless, due to the current lack of knowledge on the polychaete fauna in marinas, further studies are needed that consider other arborescent substrates attached to floating pontoons or soft bottom benthic habitats in order to fully characterize the polychaete assemblage, and therefore to assess

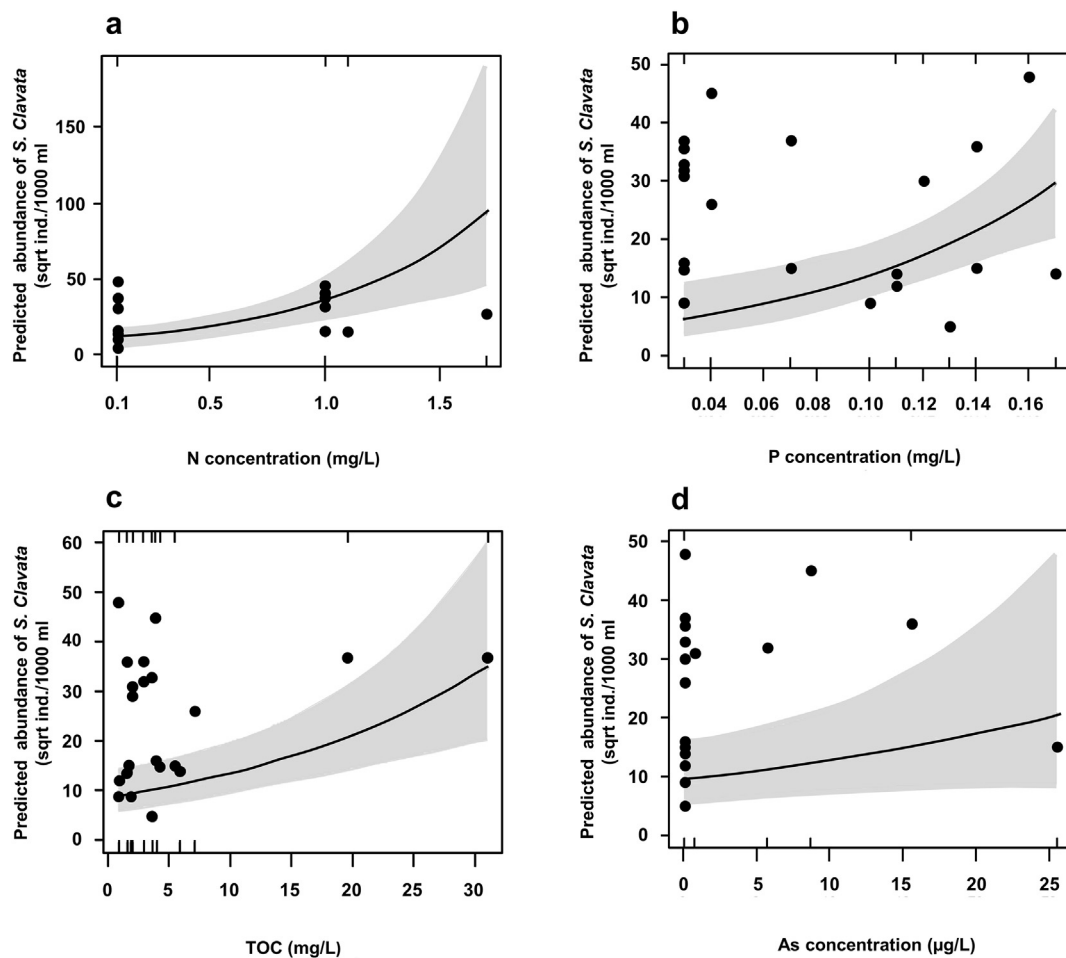


Fig. 6. GLM plots showing the predicted effect of the following significant predictors on the abundance of *S. Clavata*: (a) Nitrogen, (b) Phosphorus (c) TOC and (d) Arsenic concentration. Shading indicates 95% confidence limits for the fitted relationship.

whether the three-dimensional substrate provided by the bryozoan can house enough polychaete diversity in artificial environments.

Furthermore, coastline artificial structures are prone to be colonized by fouling taxa and translocations of non-indigenous species associated with such substrate may eventually occur (Piola and Johnston, 2008), which usually leads to impoverishment and homogenization of the biota (McKinney and Lockwood, 1999). Nevertheless, although Ros et al. (2015) recently evidenced the potential of *B. neritina* to translocate small invasive crustacean in marinas, in the present study we did not detect the presence of non-indigenous polychaete species. However, the distribution ranges of many native small-sized polychaetes are not well known. Additionally, some polychaetes species found in *B. neritina* present wide distribution and probably represent complex(es) of cryptic species (Westheide and Schmidt, 2003; San Martín, 2005). Therefore, it is difficult to fully assess whether truly exotic species are present on the studied marinas.

#### 4.2. Relationships among environmental pollutant and epibiont polychaete community

As happens in natural habitats, abiotic factors also play a major role in determining patterns of macrofauna distribution and abundance in artificial coastal habitats (Chapman and Wang, 2001). Among those factors, organic matter enrichment and heavy metal concentrations have been reported to be the most relevant factors to structure polychaete assemblages in disturbed environments (Rygg, 1985; Warwick, 2001; Dauvin, 2008). However, the effects of such factors on polychaetes inhabiting marinas had not yet been investigated. Our results

point in the same direction as those studies carried out in other artificial structures. According to the RDA, heavy metals such as Zn and Cu, and nutrient enrichment, measured as levels of N, P and TOC, were the most relevant factors to explain the structure of the polychaete assemblage in marinas; furthermore, BIO-ENV analysis showed similar results.

Disturbances in marine artificial structures usually tend to favour small-sized opportunistic polychaete species that are more tolerant to pollution (Pearson and Rosenberg, 1978; Giangrande et al., 2005). In the present study, most of these species are eurytopic organisms widely distributed in other marine habitats (Sánchez-Moyano et al., 2002; Casu et al., 2006; Melero et al., 2017). This low specificity for the habitat provided by *B. neritina* suggests that while the identity of the basibiont is important for the diversity of the polychaete assemblage, site-related environmental factors are more relevant in determining a particular assemblage of epibionts (Melero et al., 2017). Furthermore, some of these species have been previously categorized as stress-tolerant organisms. For instance, Bellan (1980) considered *P. dumerilii* to be a good biological indicator of contamination due its high abundance in contaminated waters. Our results show that *P. dumerilii* was only distributed in marinas from the Mediterranean Sea and the Strait of Gibraltar. The abundance of this species was strongly related with higher values of temperature, N, P and heavy metal concentration, while it was totally absent in the less polluted Atlantic marinas. Furthermore, *C. cirratus* is another example of a polychaete species associated with pollutant environmental areas. In fact, some authors have traditionally considered *C. cirratus* as tolerant to pollution sources in many benthic habitats (Gray and Mirza, 1979; Mclusky et al., 1980) and its tolerance to heavy metals has previously been tested under laboratory conditions

(Won et al., 2008). In the present study, according to the RDA analysis, heavy metals, TOC and P concentration were the variables which best explained the distribution of *C. cirratus* along the studied area. All these facts highlight the overall usefulness of certain polychaete taxa to be used as sentinel species in the monitoring of environmental pollutants (Surugiu, 2005). However, further studies are still needed to definitively implement the use of epibiont fouling polychaete as an effective tool in environmental management for the detection and evaluation of anthropogenic pollutants in marinas.

Our study also provided information on the ecological requirements of some polychaete species. For example, the syllid *E. naidina* has been considered a species that shows a greater presence in areas subjected to low anthropic pressure (Cardell et al., 1999; Doğan et al., 2005). However, Çinar (2003) found the presence of this species in a polluted environment for the first time. Our study highlighted that *E. naidina* is also present in the fouling communities of marinas with high levels of heavy metals, N, P and organic matter. Nevertheless, one of the most unexpected findings was the presence of *S. clavata* in marinas. This species has been reported among the dominant taxa in more pristine natural habitats such as macroalgae in shallow water habitats, mussel beds, or on hard intertidal substratum (Cinar and Gönlügür-Demirci, 2005; Casu et al., 2006; Frascchetti et al., 2006; Surugiu and Novac, 2007). *S. clavata* was thought to display high sensitivity to disturbed environments, even disappearing from highly impacted areas (Giangrande et al., 2005; Surugiu and Feunteun, 2008; Surugiu, 2009). However, our study shows the opposite behavior; in fact, *S. clavata* was the dominant species along the whole studied area. Moreover, the RDA suggests that this species showed preference for marinas characterized by higher concentrations of heavy metals and nutrient enrichment.

#### 4.3. Environmental factors and distribution of *S. clavata*

Distribution patterns of polychaetes are usually conditioned by the effect of the environmental variables intrinsic to the considered habitat (Méndez, 2002; Guerra-García and García-Gómez, 2004; Brito et al., 2005). Due to the current lack of knowledge on the occurrence and abundance patterns of *S. clavata* in marinas, this species was modeled by the enforcement of different GLMs. Among the environmental factors measured in the studied marinas, the best presence/absence model for *S. clavata* suggests that temperature and turbidity were the main variables involved in its occurrence. In fact, temperature is one of the environmental variables most relevant to explain polychaete distribution (Lardicci et al., 1993; Kuş and Kurt-Şahin, 2016). Although the macrofauna assemblage is likely adapted to withstand stressors such as high temperature values in shallow waters (Cosentino, 2011), an excessive local increase in temperature could lead to a drastic reduction in the presence of some species. This paradigm could also be reflected in some epifauna species associated with the marinas' fouling communities. For instance, our best predictive occurrence models showed that the probability of *S. clavata* occurrence decreases in marinas that reach temperatures higher than 22 °C. In addition, taking into account that salinity was highly correlated with temperature, it is likely that *S. clavata* displays similar predictive values to those registered for the temperature.

Likewise, turbidity also seems to be an important factor in explaining the presence of *S. clavata* in marinas. Both turbidity and salinity have been proposed as important variables to explain the distribution of other small invertebrates associated to *B. neritina* in marinas, such as caprellid amphipods (see Ros et al., 2015). Turbidity also plays an important role in the spatial distribution of intertidal flora and fauna (Guerra-García et al., 2006). The binomial model performed showed that the lowest predicted values of *S. clavata* presence occur in marinas characterized by higher turbidity levels. Although more conclusive data is not available for marinas, high rates of sedimentation have been proposed as the most important factor involved in the impoverishment of the vagile polychaete assemblage on hard substrates

(Airoldi, 2003). For this reason, future manipulative studies could be key to better understanding to what extent the sedimentation increase on the sessile communities in marinas could be disturbing the epifaunal associated with them.

On the other hand, and according to the negative binomial models, the abundance of *S. clavata* in relation to temperature confirmed the trend observed for the presence/absence model. Moreover, the ten best negative binomial models (conditioned by presence) partially reaffirm the results obtained both by the RDA for this species and by BIO-ENV for the whole polychaete assemblage. In summary, the highest predicted values of *S. clavata* abundance occurred at marinas where concentration of nutrients and heavy metals were highest. Furthermore, symptoms of nutrient enrichment due to high concentration of N and P in the water column seem to be the most significant factors involved in the increase of *S. clavata* abundance. Effects of these stressors often translate to impoverished polychaete faunas and the dominance of a few opportunistic organisms (Omena et al., 2012; Weis et al., 2017). Although syllids are usually related to more pristine habitats, there are documented examples where some syllids are considered opportunistic species, whose abundances increase with increasing environmental stress (Bellan, 1980; Giangrande, 1988). However, although *S. clavata* was by far the dominant polychaete species in the studied area, it is still unknown whether this pattern maintains over time or, alternatively, shows seasonal fluctuations in abundance.

Thus, to definitely check if *S. clavata* could be considered another opportunistic syllid species in the studied marinas, additional studies based on an appropriate experimental design are needed. These would be able to clarify if all the characteristics recorded by our models could make *S. clavata* able to out-compete other species and, therefore, increase its abundance in conditions of anthropic disturbance.

## 5. Conclusions

The relationships between the epibiont polychaete assemblage and environmental pollutants have been poorly explored in marinas until now. This study contributes to improve our still limited knowledge on the ecological requirements involved in the structuring of the polychaete assemblage in these novel artificial habitats. We suggest that abiotic factors such as heavy metals and nutrient enrichment were the variables that best explained polychaete distribution along the studied area. Moreover, our analysis provides the first scientific evidence of establishment of *S. clavata* populations in an artificial habitat subjected to anthropogenic disturbance. Finally, our findings highlight the need to carry out future experiments to further investigate the patterns observed in this study.

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