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High Species Richness of Decapod Crustaceans on an Urban Rocky Shore Beach

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Abstract: The rocky intertidal shore of La Caleta, an urban beach located in Cádiz (SW Spain), was surveyed for decapod crustaceans over a year. Samplings were taken monthly (March 2015 to February 2016) in three differentiated zones during the spring low tides of every month at five sites, differentiated according to the hydrodynamic regimes and intertidal levels. A qualitative sampling (present/absent) was carried out by visual identification (minimally invasive), and only those specimens with uncertain identification were collected by hand, studied at the laboratory, or identified using their DNA barcode. A total of 44 species were identified. Comparison with species richness of decapod crustaceans in other Atlantic and Mediterranean intertidal or subtidal ecosystems (including protected areas) shows higher values in La Caleta, a surprising fact considering the type of habitat and its placement (an urban and unprotected shore).

Keywords: crustacea; decapoda; biodiversity; rocky intertidal; La Caleta beach; southwestern European coast

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1. Introduction

Decapod crustaceans are an incredibly diverse group comprising more than 14,300 extant species worldwide [1]. Specifically, 431 species have been recorded in the Iberian Peninsula (2.9% of the total amount in the world and more than 65% of the European species) [2]. This abundance of species is the result of a privileged geographical location, which covers different biogeographic provinces [3]. The current study was carried out on a beach in the Gulf of Cadiz, a biogeographically interesting area due to its proximity to the Strait of Gibraltar, where the exchange between Atlantic and Mediterranean waters occurs, which favors a high biological diversity and the confluence of many species from the adjacent areas (the Mediterranean Sea and North African Atlantic coasts) [4].

Decapod crustaceans of the Iberian Peninsula have been studied thoroughly over time [5–19]. However, the largest effort to catalog decapod biota has been made in Mediterranean waters, while fewer studies have been carried out on the Atlantic coasts [20], and those have focused on a delimited biotope, such as the rocky intertidal shores are even less.

Those few works that analyse the carcinofauna of the rocky shores in different regions all over the world [21–27] emphasize the lack of catalogues of crustacean biodiversity.

It has not been possible to know the decapod biota in these specific environments of the southwestern European coasts precisely until now. Therefore, it was especially interesting to catalog the current decapod species richness of the rocky intertidal environment of La Caleta beach in Cádiz, which might be useful to evidence the degree of conservation of such an unprotected urban beach.

2. Materials and Methods

2.1. Area of Study

Flanked by the Castillo de Santa Catalina and Castillo de San Sebastian, the touristic beach of La Caleta is located in the historic district of Cádiz. These two fortifications were built on a natural rock barrier, which delimits the area studied. The intertidal environments of the Atlantic Ocean are affected by tides and are immersed during each high tide, twice a day.

Five sample sites were established along the rocky intertidal zone (Figure 1). All the existing environments of the rocky intertidal habitats were studied, separating the locations according to the degree of exposure to waves and currents to detect the higher number of decapod species and differences with respect to habitat. Three main zones, equaling an area of 0.113 km^2, were established in the zone of the two rocky barriers that surround La Caleta ("Castillo de San Sebastian" (CS) and "Castillo de Santa Catalina" (CC)), which are sheltered areas with a calm hydrodynamic regime. Here, the substrates have hard bottoms, characterized by a large number of tide pools filled with algae and boulder stones, as well as some sediment accumulations with grain sizes ranging from pebble to fine sand. A third zone (E) is represented by an exposed area with a turbulent hydrodynamic regime, which is situated on the left side of Castillo de San Sebastian; in contrast with the previous ones, this site presents a more homogeneous geomorphology due to erosion and is characterized by the absence of algae and stones. In addition, because of their considerable sizes, the CS and CC sample sites were divided into upper (U) and lower (L) intertidal zones. In this way, it is possible to distinguish between the zones that spend larger periods out of the water during each low tide from those that are only exposed for short times (Figure 1, Table 1). This rocky shore is subject to semidiurnal mesotides (tidal range 1 to 3.5 m). It is important to remark that the CS and CC sites delimit a sandy beach, but this is not considered in this study, which is only focused on the rocky environments.

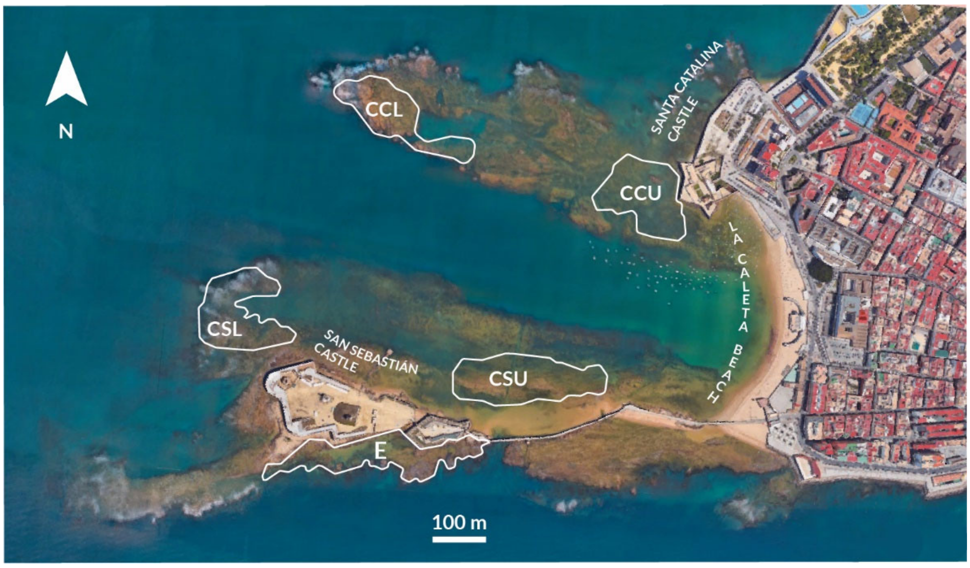

Figure 1. Map of La Caleta beach. Sampling areas delimited by white lines. Abbreviations: CCL, Santa Catalina Castle's rock barrier, lower intertidal zone; CCU, Santa Catalina Castle's rock barrier, upper intertidal zone; CSL, San Sebastian Castle's rock barrier, lower intertidal zone; CSU, San Sebastian Castle's rock barrier, upper intertidal zone; E, Exposed zone. Edited from Google Earth.

Table 1. Sampling zones, including coordinates, description of substrate types, and extension. Abbreviations: CCL, Santa Catalina Castle's rock barrier, lower intertidal zone; CCU, Santa Catalina Castle's rock barrier, upper intertidal zone; CSL, San Sebastian Castle's rock barrier, lower intertidal zone; CSU, San Sebastian Castle's rock barrier, upper intertidal zone; E, Exposed zone.

Zone	Coordinates	Substrate	Area (km^2)
CCL	36°32′02.4″ N/6°18′54.0″ W	Hard bottom with boulder stones. Heterogeneous geomorphology. Abundant pools and algae.	0.022
CCU	36°31′58.1″ N/6°18′36.9″ W	Hard bottom with boulder stones, Heterogeneous geomorphology. Abundant pools and algae.	0.024
CSL	36°31′47.0″ N/6°19′04.1″ W	Hard bottom with boulder stones. Heterogeneous geomorphology. Abundant pools and algae.	0.025
CSU	36°31′43.3″ N/6°18′40.2″ W	Hard/Sandy bottom with boulder stones. Heterogeneous geomorphology. Abundant pools with a low number of algae.	0.023
E	36°31′38.3″ N/6°18′54.5″ W	Hard/Sandy bottom. Homogeneous geomorphology. Few pools with a low number of algae.	0.019

2.2. Sampling Methodology

Samplings were performed monthly (from March 2015 to February 2016) in spring tide conditions during the low tide and the daylight. Considering the time limit of each sampling, conditioned by the period of time the rocky platform is exposed (approximately a maximum of four and a half hours), three consecutive days of sampling per month were needed in order to apply the same sampling effort in each zone. All samplings were carried out by the first author and, in some cases, accompanied by the third author. The sampling consisted of walking along a transect (during the low tide, from 3 to 4.5 h) in a straight line starting in the upper zone and finishing at the lowest zone of each sampling area. A qualitative and minimally invasive sampling methodology was used, writing down the presence of the visually localized species after checking under rocks, rock pools, among the seaweed, and collecting by hand or hand net only those individuals that could not be identified with certainty in field conditions. In these last cases, the samples were transported to the laboratory, photographed (see examples in Figure 2), and preserved in ethanol 70% for further molecular studies if needed. The dates on which ovigerous females were observed were also recorded. All the preserved specimens were deposited in the Crustacean Collection (CRUST-IEOCD) at Cadiz Oceanographic Centre (IEO-CSIC) under the catalog codes IEOCD-ICMAN/3522 to 3572.

2.3. Morphological Identification

The visual identification in the field was based on the external morphology of the specimens, using mainly the keys for the Iberian carcinofauna [7,28]. Any individuals that were difficult to identify in the field were studied at the laboratory using a stereomicroscope Leica MZ6, and the keys cited above as well as others for specific genera, such as *Maja* (Lamarck, 1801) [29], *Hippolyte* (Leach, 1814) [30], *Macropodia* (Leach, 1814) [31,32], and *Athanas* (Leach, 1814) [33].

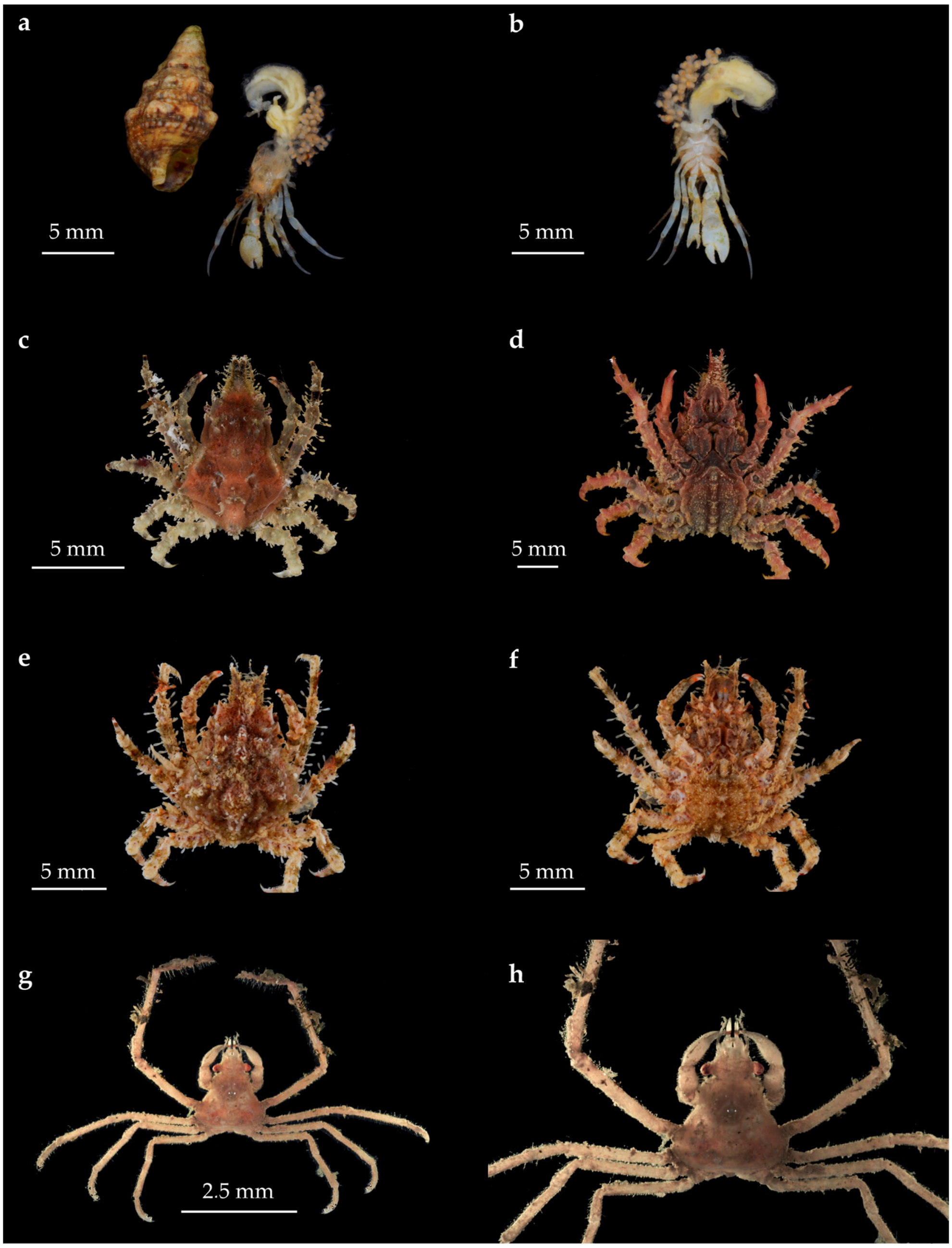

Figure 2. Specimens of decapod crustaceans collected at La Caleta Beach. *Cestopagurus timidus* (Roux, 1830), ovigerous female, (**a**) dorsal view, (**b**) ventral view. *Pisa nodipes* Leach, 1815, (**c**) dorsal view, (**d**) ventral view. *Pisa carinimana* Miers, 1879, (**e**) dorsal view, (**f**) ventral view. *Inachus gaditanus* García Raso, González-Ortegón, Palero and Cuesta, 2022, (**g**) dorsal view, (**h**) magnification of carapace in dorsal view.

2.4. Molecular Identification

For those species in which the use of morphological identification was not conclusive (or needed confirmation), DNA barcoding techniques were used.

Total genomic DNA was extracted from the muscle tissue from one pereiopod, following a modified Chelex 10% protocol [34]. Target mitochondrial DNA from the 16S rRNA gene was amplified with polymerase chain reaction (PCR) using the following cycling conditions: 2 min at 95 °C, 40 cycles of 20 s at 95 °C, 20 s at 48 °C, 45 s at 72 °C, and 5 min at 72 °C. The primers 1472 (5′-AGA TAG AAA CCA ACC TGG-3′) [35] and 16L2 (5′-TGC CTG TTT ATC AAA AAC AT-3′) [36] were used to amplify 545 bp of 16S rRNA. PCR products were sent to Stab-Vida Laboratories to be purified and bidirectionally sequenced. Sequences were edited using the software Chromas version 2.0. The final DNA sequences obtained were compared with those from specimens of Iberian brachyuran crabs obtained in the context of the MEGALOPADN project, and a BLAST search was also executed at the NCBI webpage to obtain the sequence that matched best. In the case of the *Hippolyte* species, sequences were sent to Dr. Fernando Mantelatto from the University of São Paulo to compare with his unpublished sequences of this genus.

2.5. Statistical Analysis

A multidimensional scaling (MDS) ordination based on qualitative data (presence/absence) and the Bray–Curtis similarity index was used to analyze the spatial relationships of the species observed in the study area.

Analysis of the differences between the zones that pass larger periods out of the water during each low tide from those that are only exposed for short times was carried out using MDS ordination, distinguishing between samples from CC, CS, and E, and the results were tested using a two-way ANOSIM, with exposition degree and zones as factors. An MDS ordination was made by differentiating between the U and L zones in CC and CL areas to verify the effect of the vertical gradient of stress due to time of exposition in each low tide over the species composition. In area E, there is no L zone; for this reason, it was not included in this analysis. Significant differences were tested using a two-way ANOSIM, with L/U and CC/CL as factors.

Additionally, a SIMPER analysis was carried out to calculate the contribution of each species (%) to the dissimilarity between the different areas studied. In the MDS ordination graphics, the vectors have been superimposed, indicating the correlation of the different species with the first two axes of the analysis. To show the magnitude of these correlations, the vectors have been enclosed with a circle corresponding to the maximum correlation of 1.

The statistical analyses were carried out using PRIMER v6 software [37].

3. Results

3.1. Species Richness of Decapod Crustaceans

A total of 44 decapod species were recorded in this study, belonging to 33 genera and 24 families (Table 2). The most diverse and numerous group was the infraorder Brachyura, with 24 species (16 genera), and the best-represented genera were *Macropodia* Leach, 1814, *Pisa* Leach, 1814, and *Xantho* Leach, 1814, with 3 species each one. Dendobranchiata Spencer Bate, 1888 was the taxa that presented fewer species.

The most representative species of the rocky intertidal area of La Caleta beach, those recorded all throughout the year and at all the sampling sites, were *Xantho poressa*, *Porcellana platycheles*, *Pisidia longimana*, *Palaemon elegans*, *Pagurus anachoretus*, *Pachygrapsus marmoratus*, *P. transversus*, *Clibanarius erythropus*, *Cestopagurus timidus*, *Pilumnus* sp., and *Pisa tetraodon*.

Among the 44 species, only 1 was identified at the genus level: *Processa* sp. Moreover, a species of *Pilumnus* was identified as *Pilumnus* sp. because it is a new species undergoing the description process (Schubart, personal communication). The molts of two species were also collected: one of *Atlecyclus rotondatus* (Olivi, 1792) and three of *Upogebia pusilla* (Petagna, 1792), but these species have not been included on the list because they were not collected alive. Probably they live in the subtidal zone (or in galleries in the sand–mud substrate in the case of *U. pusilla*), and their molts were deposited in the rocky intertidal zone by the currents. The species *Afropinnotheres monodi* was collected inside one of its known hosts, the mussel *Mytilus galloprovincialis* Lamarck, 1819.

A total of 14 species (*Achaeus gracilis*, *A. cranchii*, *Alpheus dentipes*, *Athanas nitescens*, *Eualus cranchii*, *Hippolyte inermis*, *H. leptocerus*, *Macropodia czerniavskii*, *M. longirostris*, *M. rostrata*, *Pisa carinimana*, *P. nodipes*, *Pilumnus hirtellus*, and *Pilumnus* sp.) were identified (or their identification was confirmed) using the DNA genetic marker 16S rRNA. Identifications were considered positive when sequences showed similarity values equal to or greater than

99%. All these new 16S sequences were deposited in Genbank under the accession codes OQ701315-OQ701331.

Table 2. List of the species recorded in the rocky intertidal of La Caleta beach by month. Abbreviations: X, present; X̲, presence of ovigerous females; O, month with ovigerous females according to the literature; N̲X̲, presence of ovigerous females in a month not previously reported.

Species	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Sicionidae												
Sicyonia carinata (Brünnich, 1768)												X
Hippolytidae												
Lysmata seticaudata (Risso, 1816)		X	O		O	O	OX					X
Hippolyte inermis Leach, 1815	O	O			O	O	O	OX				
Hippolyte leptocerus (Heller, 1863)	O	O		X		X	N̲X̲	X		X	N̲X̲	X
Eualus cranchii (Leach, 1817)	O	O	OX	O	OX	O	OX	O	X	X	X	N̲X̲
Alpheidae												
Alpheus dentipes Guérin, 1832	X	O̲X̲	OX	O̲X̲	OX	O̲X̲	O̲X̲	X	X	X	X	X
Athanas nitescens (Leach, 1814)	X	O̲X̲	O̲X̲	O̲X̲	O	O̲X̲	O̲X̲	X	X	X	X	X
Processidae												
Processa sp.					X							
Palaemonidae												
Palaemon elegans Rathke, 1837	N̲X̲	O̲X̲	OX	OX	OX	OX	OX	X	X	X	X	X
Palaemon serratus (Pennant, 1777)	O		O	X		OX			X	OX	O	X
Crangonidae												
Philocheras fasciatus (Risso, 1816)				X								
Callianassidae												
Callianassa pestai (Montagu, 1808)										X		
Diogenidae												
Clibanarius erythropus (Latreille, 1818)	X	X	OX	OX	OX	OX	X	X	X	X	X	X
Paguridae												
Anapagurus pusillus Henderson, 1888												X
Cestopagurus timidus (Roux, 1830)	X	OX	OX	N̲X̲	O	OX	O	X	X	X	X	
Pagurus anachoretus Risso, 1827	X	X	OX	O̲X̲	OX	OX	OX	OX	X	X	X	X
Galatheidae												
Galathea squamifera Leach, 1814	O	O	O							X	X	X
Porcellanidae												
Pisidia longimana (Risso, 1816)	X	N̲X̲	X	X	X	X	X	X	X	X	X	N̲X̲
Porcellana platycheles (Pennant, 1777)	N̲X̲	N̲X̲	X	X	OX	OX	OX	N̲X̲	N̲X̲	N̲X̲	X	N̲X̲
Ethusidae												
Ethusa mascarone (Herbst, 1785)		O			O	O	OX	O				
Pirimelidae												
Pirimela denticulate (Montagu, 1808)	O	O	X									
Polybiidae												
Liocarcinus navigator Herbst, 1794	O	XO	O	O	O	O	X					O
Portunidae												
Carcinus maenas (Linnaeus, 1758)	O			X				X			O	O
Xanthidae												
Xantho hydrophilus (Herbst, 1790)	OX	OX	OX	OX	O	X	X		X	X	X	X
Xantho pilipes A. Milne-Edwards, 1867	X	OX	OX	OX	O	OX			X	X	X	X
Xantho poressa (Olivi, 1792)	X	X	O̲X̲	O̲X̲	OX	OX	OX	X	X	X	X	X
Eriphiidae												
Eriphia verrucosa (Forskall, 1775)	X		O	X	O̲X̲	O		X		X		
Pilumnidae												
Pilumnus hirtellus (Linnaeus, 1761)	OX	OX	O̲X̲	O̲X̲	OX	N̲X̲	OX	X	X	X	X	X
Pilumnus sp.	X	O̲X̲	O̲X̲	O̲X̲	N̲X̲	O̲X̲	X	X	X	X	X	X
Pinnotheridae												
Afropinnotheres monodi Manning, 1993											X	
Grapsidae												
Pachygrapsus marmoratus (Fabricius, 1787)	X	X	OX	O̲X̲	OX	X	X	X	X	X	X	X
Pachygrapsus transversus (Gibbes, 1850)	X	X	X	N̲X̲	X	X	X	X	X	X	X	X
Varunidae												
Brachynothus atlanticus Forest, 1957	X	X		X								X
Epialtidae												
Acanthonyx lunulatus (Risso, 1816)	X	X	OX	OX	O̲X̲	OX	O	X	X	X	X	X
Pisa carinimana Miers, 1879			X	N̲X̲		O		X		X		X
Pisa nodipes Leach, 1815		O	X		X	OX	O					
Pisa tetraodon (Pennant, 1777)	O̲X̲	O̲X̲	OX	OX	OX	O̲X̲	N̲X̲	X	X	X	OX	N̲X̲
Majidae												
Maja crispata Risso, 1827	X	O	X		X	O	O				X	X
Inachidae												
Achaeus cranchii Leach, 1817		OX	N̲X̲	OX	O	O̲X̲	OX		X	X	X	
Achaeus gracilis (Costa, 1839)	X		X	O	O	O	O				X	
Inachus gaditanus Garcia Raso, González-Ortegón, Palero and Cuesta, 2022	OX	O		X		OX			O	X	O	O̲X̲
Macropodia czerniavskii (Brandt, 1880)	X	N̲X̲				OX		X	X	X	X	X
Macropodia longirostris (Fabricius, 1775)		O		O	O						X	
Macropodia rostrata (Linnaeus, 1716)	OX	O		X	O	O	OX	X	X	X	X	X

The collection of eight specimens of *Pisa carinimana* (including an ovigerous female) is remarkable. This represents the second record of this species in the Atlantic region of the Iberian Peninsula and the third for all of the Iberian Peninsula. Moreover, it is the first intertidal record of this species that is commonly collected in relatively shallow waters to 100 m [38,39].

When this study was carried out, the specimens of the genus *Inachus* Weber, 1795 were identified as *Inachus phalangium*. However, these specimens were studied recently in the context of another work where they were assigned to a new species, *Inachus gaditanus* [40].

3.2. Spatial Study of the Decapod Crustacean Communities

The results of the MDS ordination, considering the species observed in the three areas (CC, CS, and E) and in the samples of the 12 months, show partial segregation of the samples in the first axis, with the samples of area E grouped on the left and those of the areas CC and CS mixed together on the right (Figure 3). The vectors of this first axis point to a higher correlation of area E with *Acanthonyx lunulatus*, and areas CC and CS with *Pisa nodipes*.

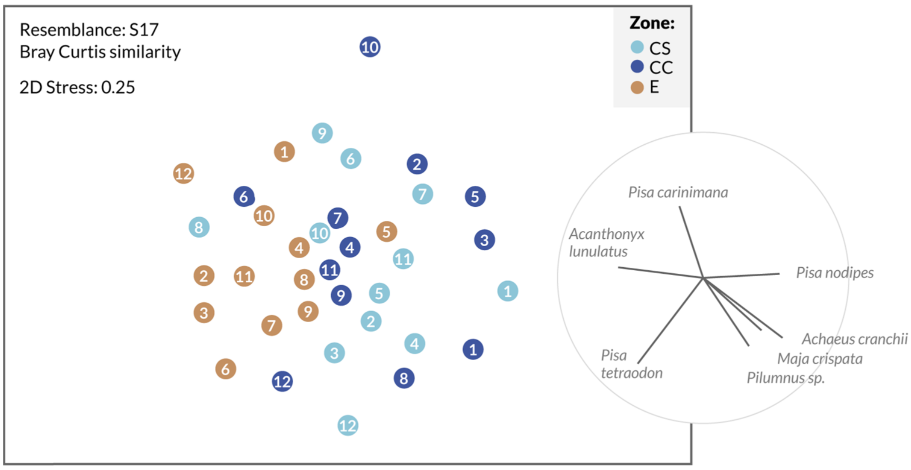

Figure 3. MDS ordination, considering all species observed at La Caleta beach in the three sampled zones (CS: Santa Catalina Castle, CS: San Sebastian Castle, and E: exposed zone) in the 12 months sampled.

The results of the one-way ANOSIM analyses indicate that the differences observed in the MDS ordination (Figure 2) for the spatial factor are statistically significant between zones CS and E ($p < 0.01$) and CC and E. ($p < 0.01$) but not between the samples from zones CS and CC ($p > 0.05$).

SIMPER analyses have provided both the most characteristic species of each zone and those that contribute to the dissimilarity between the different areas studied, specifically, between the environments subject to low exposure, grouping sampling points from CS and CC, and the most exposed environments at point E (see Table 3). The species *Acanthonyx lunulatus*, *Athanas nitescens*, *Hippolyte leptocerus*, *Pisa tetraodon* and *Pisa carinimana* (more frequent in zone E), and *Xantho hydrophilus*, *Achaeus cranchii*, *Macropodia rostrata*, *Xantho pilipes*, *Macropodia czerniavskii*, and *Eualus cranechii* (most frequent in zone C) are those that contribute to the greatest degree to the differentiation between the communities of both zones.

Table 3. Results of the SIMPER analysis for determining the species characteristic of each zone and contribution to the dissimilarity between the different areas studied: C (Santa Catalina Castle and San Sebastián Castle grouped) and E (the exposed zone). Abbreviations: Av. Ab.: Average Abundance; Av. Dissim.: Average Dissimilarity; Av. Sim.: Average Similarity; Contrib: Contribution; Cum: Cumulative Contribution; Dissim/SD: Dissimilarity Standard Deviation; Sim/SD, Similarity Standard Deviation.

Group C
Average Similarity: 74.05

Species	**Av. Ab.**	**Av. Sim.**	**Sim/SD**	**%Contrib**	**Cum%**
X. poressa	1.00	6.64	8.69	8.97	8.97
P. platycheles	1.00	6.64	8.69	8.97	17.93
P. longimana	1.00	6.64	8.69	8.97	26.90
P. elegans	1.00	6.64	8.69	8.97	35.86
P. anachoretus	1.00	6.64	8.69	8.97	44.83
P. transversus	1.00	6.64	8.69	8.97	53.79
P. marmoratus	1.00	6.64	8.69	8.97	62.76
C. erythropus	1.00	6.64	8.69	8.97	71.73
C. timidus	1.00	6.64	8.69	8.97	80.69
Pilumnus sp.1	0.88	4.90	1.75	6.62	87.31
P. tetraodon	0.67	2.78	0.87	3.75	91.06

Group E
Average similarity: 79.28

Species	**Av. Ab.**	**Av. Sim.**	**Sim/SD**	**%Contrib**	**Cum%**
X. poressa	1.00	7.01	9.62	8.84	8.84
P. platycheles	1.00	7.01	9.62	8.84	17.68
P. longimana	1.00	7.01	9.62	8.84	26.52
P. elegans	1.00	7.01	9.62	8.84	35.35
P. anachoretus	1.00	7.01	9.62	8.84	44.19
P. transversus	1.00	7.01	9.62	8.84	53.03
P. marmoratus	1.00	7.01	9.62	8.84	61.87
C. erythropus	1.00	7.01	9.62	8.84	70.71
C. timidus	1.00	7.01	9.62	8.84	79.55
Pilumnus sp.1	0.83	4.67	1.44	5.89	85.43
P. tetraodon	0.83	2.57	1.44	5.77	91.20

Groups C and E
Average dissimilarity: 26.65

Species	**Group C Av. Ab.**	**Group E Av. Ab.**	**Av. Dissim.**	**Dissim/SD**	**%Contrib**	**%Cum**
A. lunulatus	0.00	0.58	1.97	1.16	7.38	7.38
A. nitescens	0.00	0.58	1.94	1.17	7.27	14.65
X. hydrophilus	0.46	0.33	1.65	0.96	6.19	20.84
A. cranchii	0.38	0.25	1.45	0.87	5.44	26.28
H. leptocerus	0.13	0.42	1.43	0.87	5.35	31.63
P. tetraodon	0.67	0.83	1.37	0.79	5.16	36.78
M. rostrata	0.38	0.08	1.33	0.80	4.99	41.77
X. pilipes	0.33	0.08	1.23	0.74	4.61	46.38
M. czerniavskii	0.33	0.08	1.19	0.75	4.46	50.84
E. cranchii	0.33	0.00	1.14	0.70	4.26	55.10
P. carinimana	0.17	0.25	1.09	0.70	4.08	59.19
E. verrucosa	0.21	0.17	1.03	0.66	3.87	63.06
P. hirtellus	0.17	0.17	0.92	0.62	3.45	66.51
P. serratus	0.17	0.17	0.92	0.62	3.45	69.95
Pilumnus sp.1	0.88	0.83	0.91	0.57	3.40	73.35
B. atlanticus	0.04	0.25	0.85	0.61	3.21	76.56
P. nodipes	0.25	0.00	0.82	0.57	3.09	79.65
M. crispata	0.21	0.00	0.66	0.51	2.49	82.14
I. gaditanus	0.21	0.00	0.66	0.51	2.49	84.62
G. squamifera	0.17	0.00	0.52	0.45	1.94	86.56
A. dentipes	0.08	0.08	0.51	0.42	1.91	88.47
A. gracilis	0.08	0.08	0.48	0.42	1.81	90.28

The results of the MDS ordination for the samples belonging to the U and L intertidal zones show partial segregation of the samples in the first and second axes (along the increasing diagonal of both axes), leaving the samples of the upper zone grouped below and on the left and those of the lower zone above and on the right (Figure 3). The correlation vectors of the species with these two axes indicate that the species *Callianassa pestai* has a greater presence in the samples from U, while the species *Pisa tetraodon*, *Achaeus cranchii*, *Athanas nitescens*, and *Pilumnus* sp.1. are more represented in L (Figure 4).

The results of the two-way ANOSIM analyses for the intertidal factor (L or U) and zones (CS or CC) show that there are significant differences ($p < 0.01$) between the decapod crustacean communities of different heights. SIMPER analysis shows that *P. tetraodon*, *Pilumnus hirtellus*, *Athanas nitescens*, *Alpheus dentipes*, *Pilumnus* sp.1, and *Xantho hydrophilus* are the species that contribute the most to differentiating the communities from those of U because they are more frequent at L (Table 4).

Table 4. Results of the SIMPER analysis for determining the species characteristic of each intertidal zone (U: upper, L: lower) and the contribution to the dissimilarity between the different zones studied. Abbreviations: Av. Ab.: Average Abundance; Av. Dissim.: Average Dissimilarity; Av. Sim.: Average Similarity; Contrib: Contribution; Cum: Cumulative Contribution; Dissim/SD: Dissimilarity Standard Deviation; Sim/SD, Similarity Standard Deviation.

Group U **Average Similitary: 78.61**						
Species	**Av. Ab.**		**Av. Sim.**	**Sim/SD**	**%Contrib**	**%Cum**
X. poressa	1.00		8.20	7.69	10.44	10.44
P. platycheles	1.00		8.20	7.69	10.44	20.87
P. longimana	1.00		8.20	7.69	10.44	31.31
P. elegans	1.00		8.20	7.69	10.44	41.74
P. anachoretus	1.00		8.20	7.69	10.44	52.18
P. transversus	1.00		8.20	7.69	10.44	62.62
P. marmoratus	1.00		8.20	7.69	10.44	73.05
C. erythropus	1.00		8.20	7.69	10.44	83.49
C. timidus	1.00		8.20	7.69	10.44	93.92
Group L						
Average Similarity: 73.85						
Species	**Av. Ab.**		**Av. Sim.**	**Sim/SD**	**%Contrib**	**%Cum**
X. poressa	1.00		6.36	8.80	8.61	8.61
P. platycheles	1.00		6.36	8.80	8.61	17.23
P. longimana	1.00		6.36	8.80	8.61	25.84
P. elegans	1.00		6.36	8.80	8.61	34.45
P. anachoretus	1.00		6.36	8.80	8.61	43.07
P. transversus	1.00		6.36	8.80	8.61	51.68
P. marmoratus	1.00		6.36	8.80	8.61	60.29
C. erythropus	1.00		6.36	8.80	8.61	68.91
C. timidus	1.00		6.36	8.80	8.61	77.52
Groups U and L						
Average Dissimilarity: 66.26						
Species	**Group C Av. Ab.**	**Group E Av. Ab.**	**Av. Dissim.**	**Dissim/SD**	**%Contrib**	**%Cum**
P. tetraodon	0.13	0.71	2.35	1.36	8.82	8.82
P. hirtellus	0.46	0.79	1.91	1.03	7.18	16.00
A. nitescens	0.29	0.54	1.82	1.02	6.84	22.84
A. dentipes	0.46	0.54	1.80	0.99	6.76	29.59
Pilumnus sp.1	0.13	0.46	1.62	0.93	6.09	35.68
X. hydrophilus	0.17	0.42	1.60	0.88	6.00	41.69
A. lunulatus	0.25	0.38	1.51	0.87	5.65	47.34
A. cranchii	0.13	0.38	1.38	0.82	5.17	52.51
M. rostrata	0.17	0.33	1.36	0.79	5.09	57.60
X. pilipes	0.13	0.25	1.10	0.67	4.14	61.75
E. cranchii	0.04	0.29	1.10	0.66	4.14	65.88
M. czerniavskii	0.08	0.25	0.97	0.64	3.66	69.54
I. gaditanus	0.17	0.13	0.81	0.57	3.05	72.59
M. crispata	0.08	0.17	0.73	0.53	2.74	75.34
P. serratus	0.04	0.17	0.68	0.49	2.56	77.90
E. verrucosa	0.08	0.13	0.68	0.48	2.55	80.45
P. nodipes	0.04	0.17	0.66	0.49	2.48	82.93
P. carinimana	0.08	0.13	0.60	0.48	2.24	85.17
G. squamifera	0.04	0.13	0.52	0.43	1.96	87.13
L. seticaudata	0.04	0.08	0.43	0.36	1.61	88.73
C. pestai	0.08	0.04	0.42	0.36	1.59	90.32

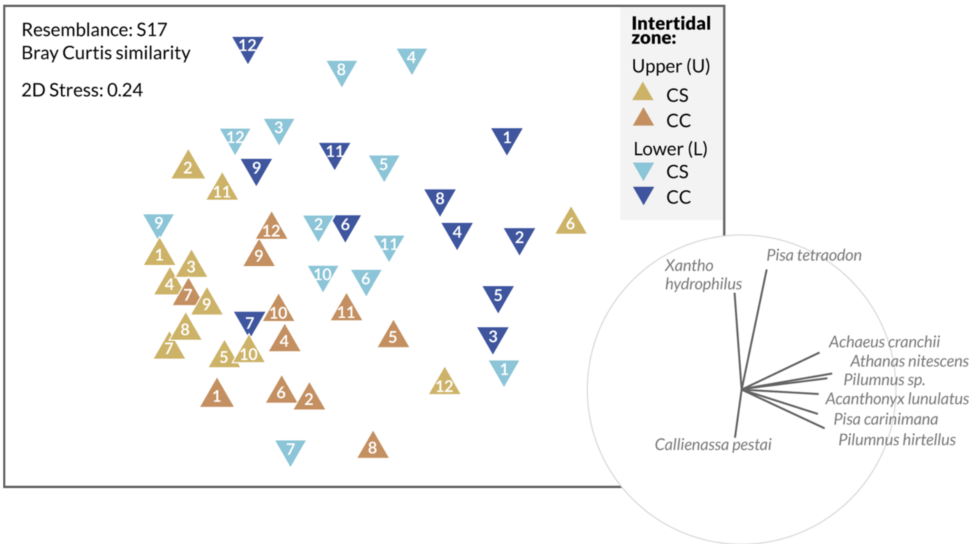

Figure 4. MDS ordination, considering all U (upper) and L (lower) intertidal samples observed at La Caleta beach in two sampled zones (CS: Santa Catalina Castle and CS: San Sebastian Castle) in the 12 months sampled.

4. Discussion

4.1. Variability of the Assemblages through the Longitudinal and Vertical Gradient

According to Velásquez et al. [41], the longitudinal gradient of surge exposure strongly affects the distribution and composition of the biodiversity of the rocky shores. The decapod assemblage of E differs from those of CS and CC, which do not differ significantly between them (see Figure 3) in this study, demonstrating the existence of two differentiated communities. The species that mostly contribute to the dissimilarities between the communities of both environments are *Acanthonyx lunulatus*, *Athanas nitescens*, *Hippolyte leptocerus*, *Pisa tetraodon*, and *P. carinimana* in the exposed site, and *Xantho hydrophilus*, *Achaeus cranchii*, *Macropodia rostrata*, *Xantho pilipes*, *M. czerniavskii*, and *Eualus cranchii* in the less exposed sites (see Table 3).

Differences between the decapod assemblages of U and L zones have also been detected (see Figure 4 and Table 4), showing that the tidal rhythm acts as an agent of stress, causing a vertical gradient (zonation) of the species richness of decapods in the rocky intertidal zone. The main difference between the L and U zones is that the species richness of L is higher and has a greater presence of *Pisa tetraodon*, *Pilumnus hirtellus*, *Athanas nitescens*, *Alpheus dentipes*, *Pilumnus* sp., and *Xantho hydrophilus* (see Table 4).

Finally, it is interesting to highlight that CCL is the site with the highest species richness, with a total amount of 34 recorded taxa (see Table 5). This could be a result of the less stressful conditions of the lower and less exposed sites, besides the rich diversity of microhabitats found within them (with a greater number of algae and a more heterogeneous substrate with irregular geomorphology and plenty of boulder stones), as well as the difficulty of access for tourists and fishermen to the CCL zone. On the other hand, a lower number of well-adapted species but with a high frequency of occurrence were found in areas CCU, CSU, and E, such as *Pachygrapsus transversus* and *P. marmoratus*, which can exploit the whole intertidal range, being dominant species in U (Table 5).

Table 5. List of the recorded species associated with the number of months and sites where they were recorded. Abbreviations: CC, Santa Catalina Castle's rock barrier; CS, San Sebastian Castle's rock barrier; U, upper intertidal zone; L, lower intertidal zone; E, Exposed zone.

Species	CCL	CCU	CSL	CSU	E
Sicyonia carinata	0	1	0	0	0
Lysmata seticaudata	0	0	2	0	0
Hippolyte inermis	1	0	0	0	0
Hippolyte leptocerus	2	0	2	0	5
Eualus cranchii	3	2	3	0	0
Alpheus dentipes	6	7	6	3	0
Athanas nitescens	8	5	4	3	7
Processa sp.	0	0	0	0	1
Palaemon elegans	12	12	12	12	12
Palaemon serratus	2	0	4	0	2
Philocheras fasciatus	0	0	0	0	1
Callianassa pestai	0	0	0	1	0
Clibanarius erythropus	12	12	12	12	12
Anapagurus pusillus	0	1	0	0	0
Cestopagurus timidus	12	12	12	12	12
Pagurus anachoretus	12	12	12	12	12
Galathea squamifera	2	1	1	0	0
Pisidia longimana	12	12	12	12	12
Porcellana platycheles	12	12	12	12	12
Ethusa mascarone	0	1	0	0	0
Pirimela denticulata	0	0	0	1	0
Liocarcinus navigator	1	0	0	1	0
Carcinus maenas	1	0	0	1	0
Xantho hydrophilus	4	1	5	2	4
Xantho pilipes	4	2	2	1	1
Xantho poressa	12	12	12	12	12
Eriphia verrucosa	1	1	1	2	2
Pilumnus hirtellus	10	7	10	4	9
Pilumnus sp.	7	2	3	0	1
Afropinnotheres monodi	0	0	0	0	1
Pachygrapsus marmoratus	12	12	12	12	12
Pachygrapsus transversus	12	12	12	12	12
Brachynothus atlanticus	1	0	0	0	3
Acanthonyx lunulatus	4	3	5	3	5
Pisa carinimana	3	1	0	1	3
Pisa nodipes	2	1	2	0	0
Pisa tetraodon	8	1	8	3	9
Maja crispata	1	0	3	3	0
Achaeus cranchii	3	2	3	0	0
Achaeus gracilis	2	0	1	0	1
Inachus gaditanus	3	2	0	2	0
Macropodia czerniavskii	3	0	3	2	1
Macropodia longirostris	0	0	0	2	0
Macropodia rostrata	4	0	4	3	1
Number of species on each sampling site	34	27	29	27	26

4.2. Biological Remarks

New information about the reproductive periods of the different species has been provided (see Table 2) in addition to previous data [7,20]. The number of months with the presence of ovigerous females has increased in this area for 11 species: *Achaeus cranchii*, *Cestopagurus timidus*, *Hippolyte leptocerus*, *Macropodia czerniavskii*, *Palaemon elegans*, *Pilumnus hirtellus*, *Pisa tetraodon*, *Pisidia longimana*, *Porcellana platycheles*, *Pachygrapsus transversus*, and *Pisa carinimana*. A remarkable case is *Porcellana platycheles*, which, according to the literature, only reproduces over three months (July to September), and now, with the present data, this has been extended by six months. All these new data records show the lack of knowledge and the scarcity of works studying the reproductive periods of decapod crustaceans, which are relevant to understand the effects of the present changes in the temperature patterns due to global warming. In some species, such as *Xantho poressa*, a tendency in the last years to start the reproductive period earlier and finish it later has been observed (Cuesta, personal observations).

Eight specimens of *Pisa carinimana* were found in the present study; this is the second record for the Atlantic waters of the Iberian Peninsula and confirms the Gulf of Cadiz as the northernmost limit of the distribution of this species so far. Further, this is the first time that this species has been observed in an intertidal rocky shore, in contrast to the literature, which associates this species with soft bottoms and shallow waters until 100 m [20,38,39]. Individuals of this species were observed during six months of the year, and one ovigerous female was found, which seems to indicate that the species is well established in this habitat in this locality.

4.3. Species Richness of Decapod Crustaceans in an Urban Rocky Shore Beach

A comparison with the results of other similar studies has been made to evaluate the species richness in the 0.113 km^2 area that form the five sampling sites of the rocky shore of La Caleta beach (Table 6). Unfortunately, most of them are not from the intertidal areas. These works also studied the number of decapod crustacean species in different geographical areas, all of them concerning the confluence of waters near the Strait of Gibraltar (except the studies located in Italy), but characterized by different amounts of stress, substrate, habitat, and degree of protection. Many of these works are not specifically on intertidal or rocky shore areas.

Intertidal rocky shores present an important number of stressful factors, such as variation in salinity and temperature, exposure to waves, desiccation during low tides, and in some cases, other additional stressors caused by humans (pollution, fishing, etc.). In the case of La Caleta beach, the rocky area suffers anthropogenic impact all year round due to both tourism and the local gatherers (shellfish gatherers and fishermen). Despite this, and surprisingly, the rocky intertidal shore of La Caleta has shown the highest species richness (44 species) of all the other low-impacted habitats with an expected better environmental status (even considering the smaller area studied in comparison with the area sampled in other studies). It has only been surpassed by the 49 decapod species recorded in the close beach of Valdelagrana (Cádiz), in *Caulerpa prolifera* meadows (see Table 4). In this latter case, the higher number of decapod crustacean species recorded could be due to the duration of the period of study (two years) and the sampling frequency (monthly), as well as the use of a trawl. Therefore, a possible correlation between the period of study and the sampling frequency with the number of different species collected could be an explanation for the results of the different studies shown in Table 4 since the rest of the studies are mainly seasonal and only for a maximum of one year.

The fact that the shallower stations, especially the intertidal areas, are more influenced by the heavy stress caused by waves and tidal currents, which also determine the number of species, must be considered. Therefore, probably enlarging the sampling zones by a few meters to deeper areas would increase the number of species considerably. Intertidal rocky shores are heterogeneous habitats, including small caves, crevices, stones, algal covers, etc., that provide shelter to an important number of different species, compared with other more uniform habitats such as algal beds. The sampling of the current study was based on a minimally invasive method, observation and gathering of the decapods by hand or hand net, and this is a less effective method than the use of other sampling devices such as dredges, trawls, or pumps, whose impacts are much greater for both the specimens collected and their habitats. For this reason, we might suppose that the real number of species in this area could be higher.

Table 6. Comparison of number of decapod crustacean species recorded in different studies. For each study, habitat, location, depth (in meters), conservation degree status (CD) of the area studied, periodicity of samples and duration of the study (P/D), sampling method (SM), number of species (N), and references are provided. Abbreviations: UB, Urban Beach; P, Present Study; NP, National Park; SCI, Site of Community Importance; SCA, Special Area of Conservation; MPA, Marine Protected Area.

Habitat	Location	Depth	CD	P/D	SM	N	Ref
Rocky intertidal	La Caleta (Cádiz, Spain)	0	UB	Monthly/1 year	Visual, hand net, and hand	44	P
Rocky intertidal	Cabo Raso and Avencas (Lisbon, Portugal)	0	NP	2 seasons (Winter and Summer)	Visual, using transect	7	[26]
Caulerpa prolifera meadows	Valdelagrana (Cádiz, Spain)	3–6	UB	Monthly/2 years	Semi-circular trawl	49	[42]
Cymodocea nodosa meadows	Mijas (Málaga, Spain)	1–5	SCI	Seasonal/1 year	Scuba divers with a manual airlift pump	34	[43]
Cymodocea nodosa meadows	Island of Ischia (Naples, Italy)	3	MPA	Bimonthly/1 year	Scuba divers with hand-towed net	18	[44]
Cymodocea nodosa meadows	Cabo de Gata (Almeria, Spain)	10–14	NP	Seasonal/1 year	Agassiz trawl with a dredge frame	34	[45]
Posidonia oceanica meadows	Mijas (Málaga, Spain)	2	SCI	Seasonal/1 year	Scuba divers using a frame (50 × 50 cm) and an airlift sampler	34	[19]
Infralitoral macroalgal beds dominated by *Halopterys scoparia*	Mijas (Málaga, Spain)	2	SCA	Seasonal/1 year	Scuba divers using a frame (50 × 50 cm) and an airlift sampler	35	[19]
Shallow sandy bottoms	Fuengirola and Marbella (Málaga, Spain)	5	SCI	Seasonal/1 year	Small heavy rock dredge with a rectangular frame	18	[46]
Fine sand bottoms	Fuengirola and Marbella (Málaga, Spain)	15	SCI	Seasonal/1 year	Small heavy rock dredge with a rectangular frame	29	[46]
Coarse bottoms	Fuengirola and Marbella (Málaga, Spain)	15	SCI	Seasonal/1 year	Small heavy rock dredge with a rectangular frame	30	[46]
Coralligenous bottoms	Fuengirola and Marbella (Málaga, Spain)	15	SCI	Seasonal/1 year	Small heavy rock dredge with a rectangular frame	41	[46]

Another possible explanation for this high species richness, despite being an urban area, could be the care taken by the tourists, shellfish gatherers, and fishermen due to the presence of policemen. The presence of local policemen in the beach area is mainly for people's security and not specifically for the protection of nature, but probably their presence serves as a deterrent. There is also surveillance of illegal shellfishing, and when it is detected, those responsible are fined, and the catch is returned to the sea. Therefore, the fact of being an urban beach could have this positive effect when compared with nearby unprotected rocky shore beaches, such as Santibañez beach (just 8 km away), located in a non-urban area and more affected by human activities [47].

5. Conclusions

The high species richness of decapod crustaceans observed at the intertidal rocky shore of La Caleta beach is due to a combination of factors. First, the habitat, since intertidal rocky shores are heterogeneous habitats, provides more options for different species. Additionally, the period and frequency of samplings (monthly during a year), as well as the sampling effort, allowed observing a higher number of species. Finally, being an urban beach could have a positive effect on the degree of protection of the habitat and species, contrary to what was initially assumed as a disadvantage compared to non-urban beaches. The sampling of the current study was based on a minimally invasive method, probably a less effective method than those used in other studies with more impact on the environment and species. Therefore, the real number of species in this area could be higher.

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